

Is this the requiem for SDI?

The Pentagon's plans for the future of SDI should carry little weight with the next US administration, which should not think of affording even the half-price version now on offer.

IT now begins to seem that the changing of the administrations in the United States in mid-January next year will spell the end of the Strategic Defense Initiative (SDI), at least in the strong sense of that term. The intended resignation (in January) of Lieutenant-Colonel James Abrahamson, the articulate cheerleader for SDI, is a substantial straw in the wind: it is unthinkable that Abrahamson's fund of personal enthusiasm for mega-projects (he was previously the one responsible for driving the shuttle programme) would have been withdrawn from a project destined to prosper as President Ronald Reagan first promised on 13 March 1983. It is also clear, from the US Department of Defense's advocacy last week of a slimmed-down version of SDI, that the essential element of the original goal — defence sufficiently near-perfect to rid the United States of the fear of a military nuclear catastrophe and thus, in principle, of the need to build offensive missile systems — is no longer regarded as feasible in the short run. But the likes of Abrahamson must also be downcast by the presidential election campaign now under way in the United States. Even Mr George Bush, the Republican candidate, is lukewarm in his declarations of support for some form of SDI. His Democrat rival, Mr Michael Dukakis, simply threatens further to cut the budget for SDI, already (at \$4,000 million a year) well below what enthusiasts would like.

That the once-proud vision of a near-perfect defence should have been so tarnished is not in the least surprising. It has been clear from the outset (see *Nature* 302, 363; 1983) that the technical goals of SDI were inherently unrealistic. Not that it is beyond the bounds of possibility to think of using infrared and other sensors to detect hostile missiles rising through the atmosphere. The difficulties have always lain in the schemes for destroying the warheads released by these missiles into ballistic trajectories, either with laser beams or mechanical projectiles mounted on space platforms. Putting paid to a single warhead would be a considerable technical feat; dealing successfully with hundreds of them, accompanied as they would certainly be by thousands of decoys, would tax not merely the ingenuity of those who would have to design the software for battle management but the financial resources of the United States, already overstretched. But the original concept of SDI was even more ambitious, with several complementary layers of defences (including terminal defence at the lowest level) working in concert. What the Pentagon now asks is that the US Congress should settle for a system consisting of two partially complete layers — some terminal defence and some interceptor missiles based in space — at half the often-quoted cost of SDI, and should regard that as some kind of bargain. But a version of SDI that is less than nearly perfect is no bargain at any price.

Terminal defences

It must be hoped that the Congress will be more sensible, and will decide otherwise. From the outset it has been plain that only two of the technical components of SDI were technically realistic — the early detection of hostile missiles by means of satellite-borne infrared sensors and terminal defence, the second of which is now on offer. But terminal defence against other people's nuclear warheads is more objectionable than it

may seem to those who argue that measures to protect civilian populations and military targets must always be wise. Because terminal defence cannot be perfect, the installation of terminal defences must be an invitation to potential adversaries to deploy hostile missiles in greater numbers. That is why the anti-ballistic missile (ABM) treaty, signed in 1972 (by the Republican Nixon administration), limited the freedom of the United States and the Soviet Union to deploy terminal defences as they saw fit; the Soviet Union kept (and has since improved) the terminal defences around Moscow, but the United States (downcast by the problems of the Sprint programme in the late 1960s) offered to forgo countervailing deployment.

Question

The United States should rest with that position. Reopening the question of whether the United States should deliberately breach the ABM treaty by deploying terminal defences and, in the mid-1990s, deploying space-based interceptors, would further hinder the negotiation of a bilateral treaty for the limitation of strategic missiles, already complicated by US insistence on its right to test SDI components as a prelude to their deployment (which would make a nonsense of the ABM treaty). That the Soviet Union may have breached the ABM rules by building a radar that could detect incoming warheads at a site in central Siberia (at Krasnoyarsk), not on the periphery of its territory, is an insufficient cause for throwing away the treaty altogether. (The Soviet government seems prepared to acknowledge its mistake, but there is no particular reason why the United States should be interested in the offer to turn Krasnoyarsk into an international space-tracking station.) To do that at this delicate stage in the deepening accommodation between the United States and the Soviet Union would be unmitigated folly. That is why the crucial question the two principal presidential candidates should be compelled to answer in the next few weeks is what importance do they attach to the remnants of SDI on the one hand and to the ABM treaty on the other?

Meanwhile, it should be noted that even the abandonment of the full-blooded SDI now in prospect does not imply that the \$20,000 million so far committed to research and development has been entirely wasted. Indeed, there is every reason why the next US administration should rake carefully through what will then be the ashes of the programme for those technical devices that will allow the design of efficient devices for spotting the launch of hostile missiles at an early stage in their trajectories. It is also likely that, whatever happens to SDI as a coordinated programme of research and development, the military in the United States and elsewhere, having had their appetites whetted, will not forget what they have learned. Nobody can complain at that. The duties of responsible military authorities everywhere require a proper regard for potential military innovation which cannot be suppressed by international agreements. But the prospect that legitimate research interests will spill over into ruinously expensive and potentially dangerous testing of devices does point to the need for a clarification of the ABM treaty. That is something to which the new US administration should bend its mind at an early stage. □



Recognizing gift-horses

A European complaint against Japanese chip-manufacturers is misguided.

WHAT is the right price for a piece of silicon whose surface has been configured as memory in a computer of some kind? Manufacturers of these devices know well enough that there is no simple answer. Chip-making is the archetypal capital-intensive business. The raw material is a material looking like a metal extracted from one of the most common materials on the surface of the Earth. Because computer memory chips are unavoidably small in size, there are few ways in which their manufacture can be guided by human hands, which are orders of magnitude larger. But the machinery required to manufacture chips is unavoidably expensive. The capital cost of a chip-making plant can easily be several times the annual salary bill of all the people it employs. The result is that the price at which a manufacturer can afford to sell his products must be very much like the answer to the question about the length of a piece of string.

This point appears not to have been appreciated in the United States by the government officials who, two years ago, negotiated the agreement between the United States and Japan on microelectronic products, which has now become a headache for the European Community as well as the United States. The agreement, much resented at the time in Europe as a kind of cartelization of international trade in microelectronics, required that Japan should not export chips to the United States at prices considered to be "too low", and that Japan should not divert cheap supplies to third markets, European countries for example. One of the consequences seems to have been that Japanese manufacturers have reduced substantially the volume of their exports of memory chips, the simplest chips, presumably because they have concluded that more complicated designs will offer a more secure market. One consequence is that computer manufacturers have been seriously inconvenienced. Another is that manufacturers and their backers in the United States and Europe are screwing up their courage to re-enter a business from which they retreated nearly a decade ago in the face of competition from Japan.

Meanwhile, nobody seems much the wiser about the proper price for a simple memory chip. In reality, in such a capital-intensive business, the price can legitimately vary within an enormous range. When demand is high and supply short, the price can be what the market will bear — usually much greater than the manufacturer needs to cover the cost of amortizing the plant that he has built. At the other extreme, when chips are abundant, the economic price need not be much greater than the cost of the purified silicon each contains. Strictly speaking, simply to break even requires that a manufacturer should collect enough revenue from the chips he sells to pay his workforce and to amortize his capital investment, which makes the economic price of a single chip a sensitive function of the volume of sales. Given the Japanese talent for making complicated machinery function efficiently, is it any wonder that Japanese manufacturers have been able to undercut their competitors elsewhere?

This is one reason why it is difficult to tell what to make of the formal complaint the European Commission is being asked to make against Japanese chip manufacturers, which may be accompanied by some form of financial sanction. Another is that the complaint apparently refers to the period 18 months ago, before Japanese manufacturers turned their attention to more complicated kinds of chips. Now, European manufacturers of computer hardware, are howling at having to pay nearly \$10 a chip for the supplies on which they can lay their hands. The moral is that people who believe that others are selling products at prices which are "too low" by their own standards should usually rejoice — provided that the trick is not accomplished by government subsidy or by breaking in some other way the rules of the General Agreement on Tariffs and Trade. □

Who pays the piper...

The public interest should not come second to the need for publication.

ROUGHLY a decade has passed since the first enthusiasms about the prospect of curing inherited defects of metabolism and other functions by the transplantation of normal genes — and many more decades may pass if the enthusiasts behave as Dr W. French Anderson is reported (on page 577) to have done last week in providing evidence for the US National Institutes of Health's Recombinant DNA Committee (RAC).

Anderson and his colleagues, who are well-known for their careful studies over many years of the candidate gene transplants with which to begin, now wish to make a gene transplantation in human subjects designed to test the viability of transplanted foreign genes rather than to rid people of a condition they have inherited. Anderson may share the opinion of many others that the RAC is making heavy weather of the issue, or may alternatively believe that, on a matter with such beneficent potential, it is more prudent that the first essays in an unavoidable direction should be cautious, with crosses on every "t" and dots on every "i". Whatever the truth, it seems to be a fact that the RAC subcommittee charged with oversight of gene transplantations believed it needed more information about Anderson's plans, postponed a decision after receiving what it considered to be inadequate information — and was then outflanked when Anderson provided the missing information orally, at the full meeting of the RAC at which the postponement would ordinarily have been confirmed.

While such a turn of events may be regarded as a sign that even very large bureaucracies are able to respond to ordinary mortals' intervention, it also betokens a measure of afterthought in circumstances in which forethought should be at a premium. But the reasons given by Anderson for his approach to the RAC committee are especially dubious: he had not provided all the information for which the subcommittee had asked for fear that it would then be published in the general press, thus prejudicing the chances that a formal account of his work would afterwards appear in some reputable scientific journal.

This is a very odd posture, the implications of which would have startled Anderson and his colleagues if they had considered them. Biomedical research, generously supported by taxpayers in the United States, is for ridding people of disease. Gene therapy is an especially promising but untried technique of which the same taxpayers happen to be distrustful. So research in the field is supported financially while would-be practitioners are required to pass through the eyes of several needles. The understanding is that the regulations will wither away when it has been shown that gene transplantation is as safe as most people expect, and effective as well.

So what will the same taxpayers make of the assertion that navigating all those needles' eyes might have prejudiced the chance of formal publication? Not much, it seems reasonable to guess. If gene therapy is for anything at all, it is for ridding patients of disease. To the taxpayers concerned, the circumstances of eventual publication are probably irrelevant. Even professional colleagues, themselves bound up in the convention that publication is a professional necessity, may share much of that view. But to offer fears that later publication will be prejudiced by full compliance with the requirements of oversight committees must damage both the prospect that research that has become contentious will be hampered and the institutions of the scientific literature, which will be accused of putting their own interests before those of the intended beneficiaries of biomedical research. The truth is that there is no responsible journal that would put its interests ahead of the public interest, as the editors of *Science* and the *New England Journal of Medicine* have unsurprisingly confirmed. If others should behave differently, the taxpayers would ask "So what?" □

Gene transfer to humans approved in face of advice

- Data not made available to expert committee
- Lymphocytes to be tracked in cancer patients

Washington

THE Recombinant DNA Advisory Committee (RAC) of the US National Institutes of Health (NIH) has approved an experiment to introduce lymphocytes containing a bacterial gene into terminally ill cancer patients. The RAC approved the experiment at a meeting on 3 October, despite the fact that an RAC subcommittee on human gene therapy had voted unanimously four days earlier to defer approval until it received additional details about the experiment. The details were provided on the day the full RAC met.

The proposed experiment was first submitted to the RAC in June (see *Nature* 333, 697; 1988). It is a joint effort between the laboratories of W. French Anderson of the National Heart Lung and Blood Institute and R. Michael Blaese and Steven A. Rosenberg of the National Cancer Institute. The experiment is intended to track lymphocytes isolated from cancer tumours which are stimulated with interleukin-2, grown in culture and then reinjected into the patient with additional interleukin-2. These tumour-infiltrating lymphocytes (TIL) cells have anti-tumour activity, and when activated can cause remarkable shrinking of some tumours.

To track TIL cells, researchers plan to insert a marker gene — in this case the gene for neomycin resistance — into the TIL cells using a retrovirus incapable of replicating itself, so that once the TIL cells are infected, no further retrovirus is produced. It will then be possible to look for evidence of the activated TIL cells in the target tumour weeks after they are injected into patients. Conventional radio labels are either too short-lived or too toxic to do the job.

The RAC referred the original proposal to its human gene therapy subcommittee, which met on 29 July. At that time, the subcommittee recommended deferring approval until several points had been cleared up. In particular, the subcommittee sought proof that a suitable mouse model for the protocol existed and that there was adequate reason to believe that the technique would insert the marker gene into the desired cell. The subcommittee also wanted reassurance that it would be possible to demonstrate that the retrovirus vector was not somehow reproducing in the patient's body.

Anderson, Rosenberg and Blaese responded to the subcommittee in a letter dated 9 September. Although it dealt with

many of the issues, the letter failed to present data that the subcommittee felt addressed their questions. On 29 September, the subcommittee held a one-hour conference call involving 10 of the 14 subcommittee members as well as three consultants. They concluded that "although some progress has been made, we [are] not yet in possession of the requested information", and recommended that the full RAC defer approval.

It therefore came as a surprise when on Monday, 3 October, Anderson appeared before the full RAC with the data the subcommittee had been requesting. Anderson says that he withheld a written response to the subcommittee because he believed that providing the material would place it in the public domain, so that it could then be published in a newspaper or other publication not subject to peer review. Anderson says that, following consultations with the editors of *Science* and *New England Journal of Medicine*, he decided that this could jeopardize publication of papers concerning this work in these journals.

Both Daniel Koshland, editor of *Science*, and Arnold Relman, editor-in-chief of *New England Journal*, agree that prior publication of data can jeopardize publication in their journals, but both felt that this was unlikely in the present circumstances. Koshland and Relman also say they would never suggest that Anderson withhold data from a legitimate government body.

Paul Neiman of the Fred Hutchinson Cancer Research Center in Seattle and a member of the subcommittee, says he felt it was inappropriate to ask the RAC to make a scientific judgement about Anderson's data without time to study them. His impression of what was presented was that it did address many of the points on which the subcommittee was seeking clarification.

Anderson says he knew that the subcommittee would be upset to learn that some of the data they were seeking were indeed available. But he insists that some experiments, including those showing that the neomycin-resistant gene could be introduced into murine TIL cells, were only completed a week before the RAC committee met.

The experiment must still be approved by the Institutional Review Boards of the two NIH institutes involved, and James Wyngaarden, director of NIH, must give his formal approval.

In addition, the Food and Drug Administration (FDA) must give its approval for the process of retroviral mediated gene transfer. The application to FDA was submitted last week. Ten patients whose cancers have not responded to conventional therapies will be the first involved in the protocol.

Joseph Palca

India plans international astronomy centre

New Delhi

ASTRONOMY teaching and research at Indian universities will benefit from plans to set up an inter-university centre for astronomy and astrophysics (IUCAA) at Pune, near Bombay. One of the main objectives of the national facility will be the development of trained manpower, and the centre will be directed by the well-known astrophysicist Jayant Narlikar.

Because only a handful of universities offer instruction in astronomy, India is facing a tremendous shortage of astronomers, says Narlikar. The subject has been neglected primarily because all existing astronomical facilities in India are located outside universities, at national observatories. The absence of a close link between the observatories and the university community has meant that even motivated students are deflected away from making astronomy their career.

The centre, set up by the University Grants Commission, will have a core of 20 leading astronomers, who will participate in research and administer the centre's

graduate school, which will be open to students from all Indian universities. The research and training activities of the centre will revolve around the giant metre-wavelength radio telescope being built about 100 kilometres from Pune.

Besides the in-house teaching programme, Narlikar says that IUCAA will promote astronomy programmes at selected universities by sending its staff on lecture tours and by sponsoring research projects. It will also run an associateship programme similar to that of the International Centre for Theoretical Physics at Trieste, Italy. The university community will be allowed access to the centre's instruments, data centre, library and computer, and the centre will help university academics to obtain observing time at observatories in India and abroad, and to develop payloads for space astronomy.

According to Narlikar, IUCAA "could very well expand its role and be a focal point for research in astronomy and astrophysics for the Afro-Asian region as a whole".

K.S. Jayaraman

US scepticism about big new cancer research campaign

Washington

A new campaign to raise \$1,000 million from public and private sources for cancer research kicked off this week in New York with a lavish dinner. But despite the welcome prospect of additional research dollars, there is concern that the STOP CANCER campaign may have unreasonable expectations about how the money will be used, as well as some scepticism as to whether Congress will be willing to play its proposed role of providing half the total by matching private contributions dollar-for-dollar.

The ambitious four-year campaign is credible because it is the brain-child of Armand Hammer, chairman of President Reagan's cancer panel. The original plan was to give the money raised to the National Cancer Institute, so that it could be used "primarily to support one of the most promising areas of research — adoptive immunotherapy". This approach for treating cancer, developed by Steven Rosenberg and his colleagues at NCI, involves obtaining lymphocytes from a cancer patient, activating them in culture with interleukin-2, and reinjecting them into the patient. An experimental protocol that involves adding a neomycin-resistant gene to these lymphocytes as a tracer for the effectiveness of this therapy was approved by the National Institutes of Health (NIH) Recombinant DNA Advisory Committee last week (see page 577).

But NCI has not been sanguine about taking such large sums of money with such specific strings attached, although it has attempted to tread carefully in dealing with Hammer's campaign. The institute's official statement about the plan — a mere six lines — says only that it would be the largest gift the NCI has ever received, that it would make possible new lines of research and that "the precise details of how the money will be used has [sic] not been finalized".

David Korn, chairman of the National Cancer Advisory Board, the body that advises the NCI director, says that many members of the board feel a formal agreement between STOP CANCER and NCI would be inappropriate. Korn met Hammer last weekend, and they agreed that any donations to NCI from STOP CANCER would be available to the institute to use as it chooses. Korn points out that it would be difficult if NCI were to make a special endorsement of the STOP CANCER fund-raising effort without giving some similar *imprimatur* to other well-meaning campaigns. But he adds that Hammer is free to stress some of NCI's research activities, for example adoptive immunotherapy, in his fund-raising

efforts. Another problem some see with the campaign is that it would provide a single-pulse of funds. Former NIH director Donald Fredrickson says such infusions of cash can perturb "the gyroscope of biomedical funding". Unless the money is put into capital projects, such as new facilities, it is difficult to use it effectively in one area, especially an area that has yet to receive general endorsement from the scientific community. Fredrickson feels that one of NIH's strengths is its ability to spread large research funds into broad support for a variety of basic sciences.

John Ultmann, director of the Cancer Research Center at the University of Chicago and chairman of the National Coalition for Cancer Research to which

STOP CANCER belongs, adds that were Hammer to succeed in raising \$500 million for NCI, Congress might be tempted to decrease its appropriation to NCI by a concomitant amount, rather than match the funds. Ultmann is sympathetic to the wish to spend more on cancer research, and he would like to see Congress increase NCI's annual appropriation by \$1,000 million as a start.

Denver Frederick, executive director of STOP CANCER, says details of how the money will be provided to NCI are still being worked out, but he expects that awards will be made on a peer-review basis. The idea, he says, is to provide NCI with the money to support grants that it has approved, but cannot now fund. Frederick says congressional leaders have been sympathetic to STOP CANCER's plans, but he believes that the campaign will have to raise some real dollars before Congress will commit to a matching scheme.

Joseph Palca

UK cancer committee protests against cyclotron grant

London

THE United Kingdom Coordinating Committee for Cancer Research (UKCCC) wants the government to withdraw a £6-million grant to a London hospital for a 60-MeV cyclotron to be used in the treatment of cancer by neutron and proton therapy. The committee, which represents the Medical Research Council, the Imperial Cancer Research Fund and the Cancer Research Campaign (CRC), says the technique is not yet proven to be better than conventional X-ray therapy. Sir Raymond Hoffenberg, president of the Royal College of Physicians and chairman of the UKCCC, is writing to the Department of Health, which awarded the grant, to ask on what the basis the decision was made and to suggest that the money be put to better use.

Dr Nigel Kemp of the CRC says there is "absolutely no evidence whatsoever" from anywhere else in the world that neutrons are any better than X-rays. The UKCCC is surprised it was not consulted before the decision was made and says the grant is "inexplicable". It makes a mockery of the coordination of research if the government is going to decide "on a whim" that neutrons are better than X-rays and agree to fund a cyclotron, says Kemp.

The grant was awarded after the prime minister, Mrs Margaret Thatcher, was lobbied on behalf of the Cyclotron Trust by Richard Packard, the eye surgeon who operated on her in 1983, and who is a member of the trust. Kemp says the grant must have been awarded on political grounds.

But Dr Mary Catterall, one of the pioneers of neutron therapy in Britain, says there is strong evidence that neutrons are better than X-rays. Those who deny that must be unaware of results from tests around the world, she says, putting criticism down to the jealousy of other researchers. Neutron therapy is more effective and more simple than x-ray therapy, "despite the fact that much of it has been carried out on low-energy cyclotrons which have had to compete against high-energy x-rays". This cyclotron will improve the treatment of cancer and keep Britain at the forefront of cancer therapy. Without one, "everybody is going to race past us." "About £50 million has been spent on cyclotrons in the United States in the past five years, says Catterall. But Dr John Antoine, associate director of radiation research at the National Cancer Institute, says research in the United States is still investigative.

The cyclotron will take three years to install and the hospital expects to be treating patients by 1992. Every year, 200–400 patients will be treated. About 3,000 people in Britain could benefit from the treatment, says Catterall. Alongside it will be a £4-million, 17-MeV cyclotron that will be used as a positron emission tomography scanner, used to study the growth of tumours and improve the understanding of how the therapy works. The government is paying £6 million of the £10.4 million capital cost of the large cyclotron and may pay 60 per cent of the running costs. Forty per cent of capacity will be reserved for private patients.

Christine McGourty

Europe evaluates its four-year plans in biotechnology

London

AN evaluation of the first two four-year programmes in biotechnology supported by the European Communities (EC) has concluded that the next programme, due to begin in 1990, should concentrate on fewer, larger projects.

While the first two programmes have been able to stimulate collaborations between laboratories in different EC countries, the size of the projects was often insufficient to attract industrial leading academic groups or the participation of industry.

The evaluation panel, chaired by Charlotte af Malborg, of the Swedish National Board for Technical Development, detected few signs that industry can be attracted to participate to a major extent in BRIDGE (Biotechnology Research for Industrial Development and Growth in Europe), the 100 million ECU (£67 million) successor to BEP (Biomolecular Engineering Programme), which ran until 1986, and BAP (Biotechnology Action Programme), which is in its last year.

While many projects in BEP and BAP have not been well defined nor worked on by a critical mass of scientists, there have been a number of successful projects, particularly in the genetics of plants and industrial microorganisms, according to the evaluators' report*.

Among the limited number of projects it recommends that BRIDGE tackles is the sequencing of the yeast genome, the construction of a detailed molecular genetic map of an economically important plant and animal, and a focused programme in protein engineering.

An annual budget of up to 0.5 million ECU should be devoted to the projects in BRIDGE to ensure that they attract high-quality participation, and the projects there should be a project manager in each case. These projects should consume 45 per cent of the budget of BRIDGE with another 25 per cent set aside for 'science-led' projects, for which proposals should be invited.

The training programmes financed in the past receive praise but need to be better marketed, says the report, particularly as they should be expanded in BRIDGE. But the evaluators believe that recipients are too generously supported, and that the level of payments should be reduced by 10 per cent to increase the travel budget.

Peter Newmark

Socialist government designates research as priority for France

Paris

"LOOSEN up, adapt and modernize". This is research minister, Hubert Curien's message to the CNRS (Centre National de la Recherche Scientifique), France's largest civil research employer, following the first ever cabinet meeting wholly dedicated to a national research organization last week. The 1989 budget gives the CNRS a long-awaited boost in terms of new jobs and money for equipment and running costs (see *Nature* 334, 556; 1988). But, even if he broke the news gently, Curien made it clear that management reforms can no longer be avoided.

The CNRS, which employs over 10,000 researchers and 15,000 technicians and administrators, currently absorbs over 20 per cent of the national civil research budget. This means that any major change in government research policy will inevitably affect the functioning of CNRS. When the Chirac government sought to cut back on public spending, while increasing the role of the private sector in research, CNRS bore the brunt of the squeeze, especially in terms of net job losses and a remit to carry out more applied research (*Nature* 329, 380; 1987). Now, it is the CNRS that benefits most from the new government's designation of research as a national priority.

Of the 919 new jobs created by Curien, 384 (284 researchers and 100 technicians) are earmarked for the CNRS. A further 427 posts will be changed as a result of regrading. By putting employment at the top of his list of priorities, Curien marks the government's diametric opposition to the previous administration's research strategy. Not only is there a return to a policy of annual recruitment (about 5 per cent per year), in order to offset the long-term effects of an unusually large number of middle-aged researchers. But Curien specifically wants to attract young people to research by raising doctoral grants to FF7000 (about \$1,100) per month. Over 100 fellowships are also to be available to encourage foreign researchers to visit French laboratories.

Other good news for CNRS is a 23 per cent increase in money to buy semi-heavy equipment, and a 5.6 per cent increase in funds for laboratory running costs. But the nation's need to balance the books, to increase its technological competitiveness and to respond to demands for greater regionalization mean that CNRS, too, must do some housekeeping.

Curien appeared to have some difficulty in putting this message across, while at the same time reassuring the CNRS committee that major reforms will not be called for. The problem is that both basic and

applied research need to be stimulated, while there must be tighter control of the quality of research and the management of resources. "There can be no applied research without the vigorous development of fundamental research of international quality", says Curien. "The CNRS therefore has the responsibility to contribute by pushing back the frontiers of knowledge. But its work must also contribute to economic and social development."

In order to encourage better productivity, Curien has asked the director general of the CNRS, François Kourilsky, to draw up and instigate "modernization measures" by mid-1989. This, he explained, will involve regular auditing and the application of new procedures to evaluate research. Curien also wants to see CNRS draw up mid-term priorities and to identify the strengths and weaknesses of French research, with the inevitable consequence that some groups who are carrying out research whose "interest has diminished" will be wound up.

Curien has regained overall control of the national research budget and has taken measures to improve coordination with other ministries, notably industry and education. A consequence of this coordination is that university research, which depends heavily upon CNRS input, will also be scrutinized. Joint CNRS-university projects have, says Curien, reached saturation and need to be pruned, albeit a painful task. He hopes that groups whose grants are cut will nevertheless be able to create "research networks" in order to keep abreast of developments. At the same time, he wants to see more researchers involved in university teaching – vocations which are largely separate.

Having stressed that the prime function of CNRS is to carry out basic research of international quality, Curien has called for "openness to the economic world". This means greater collaboration with engineering academies, which have often snubbed CNRS, and the continued development of contractual work with large companies and small businesses.

As a microcosm of the nation's research strategy, the "do everything better" message addressed to the CNRS signals that the government has sought primarily to reverse measures set up by its predecessors, leaving a definite orientation of policy, particularly regarding technology transfer, to next year.

François Kourilsky, who was appointed in July, seems to have been handed a Chinese puzzle which he is so far unable to solve. This may explain why he has remained silent, even during last week's press conference.

Peter Coles

* Evaluation of the Biomolecular Engineering Programme-BEP (1982-1986) and the Biotechnology Action Programme-BAP (1985-1989). EUR 11833, European Communities L-2985 Luxembourg. Price 11.25 ECU.

Withdrawal from telescope project ruins space plans

Sydney

Two years after the Australian Space Board was set up, Australia's National Space Program is still not off the ground. In its first two years its budget has been restricted to funding previous commitments and has dropped from A\$5.25 million in 1986–87 to A\$3.2 million in 1987–88. Now comes news that one of its key projects, the Lyman ultraviolet satellite-borne telescope, is to be abandoned.

The Lyman telescope was to be a joint project between Europe, the United States and Australia. Australia was

invited to take part because of the pre-eminence of astronomers at the Mount Stromlo and Siding Spring Observatories in developing ultraviolet detector assays. The European Space Agency (ESA) is to decide next month whether to proceed with Lyman.

The end of Australian participation in the Lyman project is a consequence of the government's reorganization of science over the past two years. When Australia first became involved in the Lyman project, there was an independent Department of Science, with an independent

minister, Barry Jones, at its head. Subsequently, the Department of Science was dismembered and its functions scattered. Many of them were absorbed by the Department of Industry, Technology and Commerce (DITAC), where Jones now serves as a junior minister.

The Space Board operates under the auspices of DITAC and its chief mission is now to encourage the development of space industry. It takes the view that expensive science projects such as the Lyman telescope are not the most cost-effective way to use Australian industrial capabilities, and that such space science would more properly be funded by a scientific grants body such as the Australian Research Council.

This year (1988–89), the Space Board's budget has been restored to A\$5.4 million, but most is earmarked for an Australian ground station to receive data from ERS-1, the European remote sensing satellite scheduled for launch early in 1990 (see page 581).

The Space Board will spend a large portion of the remainder of its budget on consultants who will prepare an Australian Space Industry Development Strategy. But some feel this money will be wasted. Ted Stapinski, general manager of Auspace which had the main contracts for the Lyman telescope project, points out that the earlier Madigan report mapped out what Australia should do in space. He describes Australia's space policy as "running around in circles without getting anywhere".

The Madigan report recommended that a space board be set up as a statutory body and that A\$500 million be spent over a period of five years. A national space programme would serve the country's needs in communications, resource management, remote sensing, surveillance and science.

Australia spends far less on space per head of population than comparable countries. In addition to the budget of the Australian Space Board, Australia will spend a further A\$5.2 million on space through its largest research body the Commonwealth Scientific and Industrial Research Organization (CSIRO). Taken together with university-based research, this gives an expenditure of about A\$0.70 per person annually. In Canada the figure is A\$5.70, Sweden spends A\$4.70, and in the United States the National Aeronautics and Space Administration alone spends A\$41 per head of population annually.

According to Stan Schatzel, who is technical director of Australia's largest aerospace company, Hawker De Havilland Ltd, and the man who conceived the Cape York Spaceport proposal, these statistics reflect the fact that the Australian government has completely failed to see the potential of space industry.

Charles Morgan

Soviet archaeological sites threatened by developers

London

THE discovery of Moscow's first birch-bark manuscript last month has again called into question the efficacy of Soviet laws on the protection of archaeological sites. Theoretically, the rules are clear; no civil engineering work in areas thought to contain archaeological remains, except by agreement with officials responsible for the protection of ancient monuments. But only last-minute intervention has rescued what seems to be an important new site.

Under the rules, even if remains come to light in what was previously thought to be virgin ground, all construction work must cease until the site has been properly excavated and recorded. But the Moscow birch-bark manuscript was uncovered in one of the most historic parts of Moscow, near the Resurrection Gate, during improperly sanctioned road repairs.

A team from the Institute of Archaeology of the Academy of Sciences of the USSR and the Moscow City Party Committee heard of the discovery and launched a rescue dig of what proved to be settlement layers dating back to the thirteenth century. The birch-bark manuscript, discovered in the fifteenth-century level, is a 16 × 3 cm fragment containing 17 lines with four or five letters in each line, written across the grain of the bark, and appears to be part of a letter.

The Soviet Union is relatively poor in historical manuscripts (the recent Catalogue of Slavonic-Russian manuscript books conserved in the USSR lists about 500 from the eleventh to thirteenth centuries). The traditional use of wood as the principal building material in Russia, and the consequent frequent and disastrous fires, meant that relatively few manuscripts survived. During the past three decades, however, some 250 birch-bark manuscripts have been found at various

sites in Russia and also Zvenigorod in western Ukraine. But it is estimated that as many as 20,000 more remain to be discovered in the Novgorod area alone — if the developers do not get to them first.

Moscow City Council is at present preparing new rules on the city's archaeology, which *Pravda* urges should not be delayed. The matter is especially urgent because reconstruction of the historic city centre is due to begin next year.

Whatever rules emerge, experience has shown that their usefulness will depend on a sufficiency of trained archaeologists to supervise excavations. But such specialists are in short supply.

The humanities, including archaeology, are low on the priority lists of the Academy of Sciences, the higher education system and the Soviet government planners. Moscow and Leningrad Universities each turn out around ten trained archaeologists a year, which would barely compensate for natural wastage, even if they all went straight into jobs in say, city planning, where archaeologists are urgently needed. In practice, the Soviet system by which new graduates repay the state for their education by serving in an assigned job for three years, means that many of these young archaeologists are sent to secondary schools, where their expertise is wasted. There is virtually no effective archaeological service, even in historic cities such as Pskov and Novgorod, to be developed as tourist centres.

The "Moscow archaeological expedition" of the Academy of Sciences, its director, Dr V. Alekseev commented in *Pravda*, "includes fewer specialists than the fingers on one hand". And while Moscow has no special archaeological service, Prague, for example, has an efficient one staffed by 40 fully trained archaeologists.

Vera Rich

British reluctance to pay tries patience of its ESA partners

London

AT next week's autumn council meeting of the European Space Agency (ESA), each member state will approve the 5 per cent annual increase in the science budget. Except perhaps Britain. Most of the ESA members are eager to get on with Horizon 2000, the centrepiece of the science programme, and are frustrated by Britain's caution. So will Britain give way? That was the question in the minds of space



Professor Reimar Lüst says UK must pay.

scientists at a seminar called "Space and the United Kingdom — *Quo Vadimus*" held at the Royal Society last week.

Professor Reimar Lüst, director general of ESA, strongly hinted to the British government that another veto on the annual increase would be unacceptable. The science programme is the "backbone" of ESA, he said. "It is essential that every member state is willing to pay its share." Each member's contribution is the annual "fee" to belong to the club, and if the majority agrees on the size of the fee, then others must fall into line.

Professor Roger Bennett, director of the science programme, said after the seminar that he expected Britain to approve the increase. "They vetoed it last time and we are not expecting them to do it again."

Job for Trivelpiece

Washington

ALVIN Trivelpiece is to leave his post as executive officer of the American Association for the Advancement of Science (AAAS) to become director of the Department of Energy's (DoE) Oak Ridge National Laboratory. Trivelpiece joined AAAS last year after six years as director of the DoE's Office of Energy Research.

Joseph Palca

The head of the British National Space Centre (BNSC), Arthur Pryor, declined to reveal the government's plans. But the space community is not optimistic. "It could come to a rather nasty crunch", said Professor Kenneth Pounds, of the University of Leicester. The other members are determined to go ahead, and they feel so strongly about it now that if Britain vetoes the increase, it might be "pushed out" of ESA. It, could be relegated to associate membership; a remarkable position for one of the founder members of the agency to be in, he said.

British space scientists are embarrassed by the government's attitude towards ESA. And at the seminar, Lord Shackleton openly apologized to Lüst for the "shocking" behaviour of Britain's ministers. He thanked ESA for its "tolerance and kindness" when Britain's ministers had been "positively offensive". Their haggling over costs may have "irreversibly damaged" Horizon 2000. Britain's partners in ESA "might prefer we lived on a different continent", said Professor David Southwood of Imperial College, London.

If Britain did veto the increase, Horizon 2000 would be stretched so far into the future that it would lose its credibility, said Bonnet, and Europe would fall behind its competitors. The whole thing would have to be "scrapped and redesigned" said Pounds, and it was not "luxurious" to start with. Even the first cornerstone, the solar-terrestrial science project, which was finalized in August (see *Nature* 334, 643; 1988), would have to be reconsidered.

The planned 5 per cent increase on the present budget of 200 million ECU, about £140 million, would increase Britain's contribution by £1 million; a "trivial" sum, says Pounds, to add to the national budget of £130 million. And the losses to the country in terms of industrial contracts and scientific opportunities would be far greater.

Britain has argued two ways of reducing its contribution. Bonnet said both were unacceptable. One was to make the science programme optional. The other was to reduce the contributions of all members as three countries, Austria, Finland and Norway, have recently joined. But funds from the new members barely cover the increase in costs caused by mission delays, said Bonnet.

If Britain relinquishes its status as full member, that could signal the beginning of the decline of its space science, said Pounds. Young researchers will go abroad and the space community will age, as it has done in the United States. And Pryor's attitude is not encouraging. Data-

processing "may be the centrepiece of what we can achieve", he said. But without a close relationship between research and theory the subject is dead, said Pounds.

Speaking at the seminar, Pryor said that the BNSC would not lead the country's efforts in space and there would be no national plan, but possibly a "chart", though what that will be remains somewhat vague. When asked how the space community could put its case better to the government, he said that ministers were not motivated solely by the "blindingly obvious". But he assured researchers that there was no "great conspiracy" against them.

Funding problems are holding back another of ESA's projects. ERS-2, the European remote sensing satellite due to take over the role of ERS-1 in 1993, is in jeopardy because Britain and France have not agreed to fund it. Of the 13 participants in ERS-1, they are the only two which have not agreed to support its replacement, but together they are due to contribute 50 per cent of the budget. Their contributions are relatively high because of the significant industrial returns they received from ERS-1. Last week, Pryor questioned this system, saying that "fin-



Lord Shackleton attacks government policy.

ancial capability does not always match technological capability".

The mission manager for the ERS satellites, Guy Dochossois, said the situation was now very difficult. The other members are keen to go ahead, but without Britain and France ERS-2 will have to be abandoned, because their expertise would be difficult to find elsewhere. If it is abandoned there will be a data gap of two to three years between the end of the lifetime of ERS-1 and the availability of data from the polar platform, ESA's main contribution to the Columbus space station programme. Lüst said that if Britain had to make a choice, it should fund ERS-2 and not the polar platform, which is over-subscribed. **Christine McGourty**

International problems in science and technology studied in Japan

Tokyo

A report issued last week by a committee of leading Japanese academics and industrialists calls on Japan to initiate large-scale international research projects and to promote the exchange of data and development of science and technology on a global scale.

The report, entitled *Summary of International Problems in Science and Technology*, was prepared by a subcommittee of the Council of Science and Technology, Japan's top science policy-making body which is chaired by Prime Minister Noboru Takeshita. The subcommittee's recommendations are intended to meet obligations under the recently signed US-Japan Science and Technology Agreement. But they are also intended to counter US moves to restrict the use of advanced technology for trade and/or military reasons.

The Human Frontier Science Program is cited in the report as an example of the type of international project Japan should initiate. And at the council meeting last week where the report was presented, Takeshita urged the heads of the Foreign Ministry and the Science and Technology Agency to make every effort to implement the Frontier programme. (The agency and the Ministry of International Trade and Industry have applied for about \$20 million in fiscal year 1989 to establish a foundation in Europe that will distribute grants and fellowships for international research on the brain and molecular recognition.)

To facilitate Japan's participation in international research projects, the subcommittee calls on the government to put more money into basic research; to establish a new system for research evaluation so that those who carry out good research are amply rewarded; to upgrade the environment of research facilities, for example by employing more technicians (technicians are noticeably absent in Japanese universities); to create a more flexible research management system that will encourage the flow of researchers between institutes within and outside Japan; and to establish world-class 'centres of excellence' in Japan.

Hiroshi Inose, director of the National Center for Scientific Information Systems, suggests in the appendix to the report that Japan should set up a large "Institute for Useless Research". The institute would award funds for research that is untied to applications, economic considerations, efficiency or success. This suggestion contrasts with developments in the West where governments are trying to tie research to industry.

To encourage the influx of foreign

scientists to Japan, the subcommittee recommends the introduction of new fellowship and workshop programmes and the establishment of Japanese language-teaching facilities both within and outside Japan. During negotiations of the US-Japan Science and Technology Agreement, one US negotiator suggested that Japan should pay all language tuition expenses for US scientists. Inose, in his personal remarks in the report's appendix, says he finds this suggestion "cute" and he thinks Japan should meet it by establishing language-training centres in major cities of the world. Inose suggests that the centres be modelled along the lines of West Germany's Goethe Institut.

Intellectual property rights were another contentious issue in negotiations of the science and technology agreement. In the agreement this problem is left to solution on a 'case by case' basis. The subcommittee report says that it has been difficult to reach international agreement on intellectual property rights because of their relationship to economic power and national security.

Japan is particularly concerned about provisions in the new US omnibus trade bill which will make it easier for the United States to impose import restrictions on technology that is judged to violate patents held by US companies and citizens. The subcommittee calls for the developed nations "to walk in pace" to reach a "new consensus" on rules for intellectual property rights.

But the issue of most concern to members of the subcommittee is US moves to restrict technology for military reasons. Both Inose and Junosuke Kishida in the appendix to the report mention a list of "military critical technology" prepared by the US Department of Defense under the recommendations of the Bucy report. Inose and Kishida fear that this list, which is classified, covers virtually all leading-edge technology including dual-use technology which can be used for both civilian and military purposes. And Inose says Japan would be in a "very difficult" position if this list is used to keep technology secret.

The council report recommends that Japan should promote the free exchange of data on a global scale and should continue to concentrate on development of science and technology in the civilian sector. And Inose urges Japanese government officials to continue to do their utmost in negotiating with the United States over the science and technology agreement so that the agreement is a "plus" for Japan. If they fail, it could be "a great disaster".

David Swinbanks

Field tests end

One of the first experiments approved in the United States involving the environmental release of a genetically engineered organism has been ended prematurely by its company sponsor. BioTechnica, a biotechnology company based in Cambridge, Massachusetts, has halted its field test of a strain of *Rhizobium meliloti* engineered to have enhanced nitrogen fixing capabilities because the microbe failed to increase the growth of alfalfa.

Despite local controversy, BioTechnica began the field test of the recombinant *Rhizobium* last spring on a plot of alfalfa at a farm owned by the company in Pepin County, Wisconsin (see *Nature* 327, 90; 1987). The bacterium contained plasmids bearing several copies of the gene for the production of the enzyme nitrogenase, and was administered to the alfalfa plants in irrigation water. By the end of the growing season, the company could not detect the presence of the recombinant organism in the soil or the root nodules of the alfalfa plants, and plants treated with the engineered *Rhizobium* grew no faster than those left untreated.

BioTechnica plans to try again next year using *Rhizobium* with extra nitrogenase genes integrated into its genome, and coated onto the alfalfa seeds or poured into the furrow as the seeds are planted. C.E.

What's in a name?



THE name of Professor Alec Boksenberg has joined the classical heroes in the sky after a ceremony at the mediaeval castle of Herstmonceux, home of the Royal Greenwich Observatory,

when an asteroid was named after the observatory's director. The asteroid, 12-15 km in diameter was discovered in 1979 by Eleanor Helin, an astronomer at the Jet Propulsion Laboratory in Pasadena. It goes under the official name of Asteroid (3205) Boksenberg 1979M06.

C.McG.

Kingsbury resigns

DAVID Kingsbury, assistant director of the National Science Foundation for Biological, Behavioral and Social Sciences, has resigned with effect from 15 October. Over the past year, Kingsbury has been the target of an investigation by the Justice Department of a potential conflict of interest regarding his relationship with a private pharmaceutical company. Kingsbury says he is resigning in order to pursue his research interests in slow viruses. He will join the faculty at George Washington University where he currently has a laboratory.

J.P.

Narmada Valley irrigation plan a test case for World Bank

New Delhi

OPPOSITION to a giant irrigation plan for the Narmada river basin is gaining momentum in India. A group of some 200 leading scientists, ecologists and administrators has presented a petition urging Prime Minister Rajiv Gandhi to scrap the Narmada Valley Project, financed by the World Bank, and to look for environmentally safer alternatives.

Signatories to the petition include Dr M. S. Swaminathan, president of the International Union for Conservation of Nature and an adviser to the World Bank. By making him their ally, Indian environmentalists hope to project the Narmada Valley Project as a test case for the World Bank's new policy of considering the environment along with economics.

The Narmada river basin covers the states of Madhya Pradesh, Maharashtra and Gujarat. The plan is to construct 30 major, 135 medium and 3,000 minor dams on the river and its tributaries.

Three major dams have already been built and about \$300 million has so far been spent on a fourth, the Sardar Sarovar dam in Gujarat. Work on the fifth dam, Narmada Sagar in Madhya Pradesh, has just begun. The project will mean eviction of an estimated one million people, including 67,000 'tribals', and submergence of 300,000 hectares of forest, containing several archaeological sites. The government's own Department of Forests has admitted that the social cost of the Narmada Valley Project will be about \$3 million.

The blueprint for the project was prepared more than 15 years ago when protection of the environment was a low priority. Swaminathan and other campaigners now believe that earlier calculations were wrong and that the water supply from the Narmada had been seriously overestimated.

They also doubt the wisdom of large-scale canal irrigation in water-retentive black soil. Irrigation from the first two dams in the scheme has caused soils to become waterlogged and crop yields to fall. A study conducted by the Indian Institute of Science for the Narmada Sagar project shows that 40 per cent of the command area will become waterlogged.

The Department of Forests has indirectly admitted that although plants totalling 2,500 MW capacity will be installed, there will be firm power generation of only 523.5 MW.

Despite opposition, the central and state governments are going ahead with the project. The Department of Forests has granted clearance for the project without waiting for the environmental risk

assessment studies the department itself had initiated. Meanwhile the World Bank has withdrawn its support for the scheme until agreement is reached on a plan to resettle the people who will be displaced by the dams.

US legislators attack greenhouse gases

Washington

A comprehensive bill aimed at slowing the build-up of greenhouse gases in the atmosphere was introduced into both houses of the US Congress last week. The 200-page bill, the work of Claudine Schneider (Republican, Rhode Island) and George Brown (Democrat, California), resurrects much of the enthusiasm for energy efficiency of the oil-shock era but with a new imperative — failure to act quickly could mean global catastrophe.

Drought, forest fires, heat waves and hurricanes in the United States this summer and disastrous floods in Bangladesh are all cited by Congresswoman Schneider as omens of the bigger disasters to come if the rise in greenhouse gases and attendant global warming remains unchecked.

Schneider believes that by focusing on more efficient use of energy, the remedies detailed in the Global Warming Prevention Act can be provided for nothing. Better energy use will mean increased US industrial productivity and provide 'cost-free' the desired fall in output of carbon dioxide, the principal greenhouse gas.

Overall, Schneider sees the bill providing energy savings of \$5,000 million per decade. Among specific measures are tax rebates to encourage consumers to buy

India's river valley projects are notorious for cost over-runs, and total cost may reach \$70,000 million — to irrigate two million hectares of land. "This works out to an investment of \$28,000 per hectare", claimed a leading article in an Indian newspaper, "making it by far the world's most expensive way of watering the land". Indian ecologists say that modern technology must be able to offer a cheaper solution.

K. S. Jayaraman

energy-efficient cars, new standards for vehicle fuel performance and a tax penalty for inefficient 'gas guzzling' vehicles. Taken together, these will ensure that average automobile fuel consumption will reach 45 miles per gallon by 1999.

Alongside fuel efficiency measures are incentives designed to spur development of renewable energy resources, to prevent deforestation and to increase reafforestation internationally. The bill even contains measures to promote universal family planning services, recognizing that population growth ultimately drives increased energy use and thus carbon dioxide output.

Schneider launched the bill in the Congress's final weeks to ensure that there is a 'blueprint for action' when Congress reconvenes and a new president takes office in January.

But although the bill enjoys support from both Democrats and Republicans, there is no guarantee that it will ever become law. In the past week, automobile interests won a reduction in fuel efficiency targets to make it easier for them to market large cars, and attempts to restart congressional debate of the Clean Air Act, which would have hit at the worst polluters, were abandoned.

Alun Anderson

Swapping power for trees in the Americas

Washington

PRESSURE groups in the United States have been quick to make fear of a global warming an ally to their various causes. Legislators espousing increased energy efficiency were first off the mark, but not far behind are proponents of nuclear power who sense that much may be made of its unique advantage — it creates energy without generating carbon dioxide. Natural-gas companies have also been beating the drum; gas-fired power stations produce just half as much carbon dioxide as those fired by coal.

One company about to open a new coal-fired power station has announced the imaginative operation of planting enough trees to fix more carbon than the plant will produce. But at a rough estimate, the whole continental United States west of the

Missouri would have to be reafforested to start reducing global carbon dioxide levels.

AES Thames, a subsidiary of Applied Energy Services, will at least be sure that its 180-MW plant being built at Uncasville, Connecticut, will make no net contribution to the level of greenhouse gases. The plant's production of 15 million tons of carbon over the next 40 years will be offset by the planting of 52 million trees in Guatemala.

In Guatemala the project is to be managed by the aid agency CARE, which will work with the Guatemalan forest service and the Peace Corps. The project was put together by the World Resources Institute which sees it as an inexpensive way to combat the problems of deforestation in developing countries while helping prevent global warming. Alun Anderson

Eliminate poverty first . . .

SIR—While the decision that under the Indo-US Vaccine Action Programme (VAP) (*Nature* 332, 198; 1988) US scientists should produce recombinant vaccines for the “most important diseases of India” may be a gesture of goodwill, there is considerable misgiving in the Indian scientific and medical community. We are concerned about the interests of our country and especially of the poor, who will be the subjects for the trial, but not necessarily the ultimate beneficiaries.

A. S. Paintal and G. S. Bhargava have rightly said that no vaccine should be tried in India unless approved for use in the United States and that India should be capable of producing its own recombinant vaccines before trials are undertaken, but experience also shows that in the free world, where multinationals eventually dictate production and pricing, India may be forced to buy these same vaccines (as it has already for rabies, measles and hepatitis B) at a high cost.

Yet the most important disease in India today is undoubtedly tuberculosis, for which BCG vaccine as well as cheap and highly effective drugs are available. There are 9 million cases annually of whom 400,000 die. Tetanus, for which we have had a cheap and effective vaccine for several decades, still claims 240,000 lives each year. An effective triple antigen vaccine is also available, but has not reached the majority of our people. The control of diarrhoea lies in the provision of water supply and sanitation, and ORT is an adequate standby, yet this new programme is aimed in part at controlling that. Diarrhoea is caused by a number of viral and bacterial agents which vary in space and time. Will there be a polyvalent vaccine for all these? Respiratory infections of childhood are due to poor housing and are caused by a variety of organisms. Will that mean another polyvalent vaccine because we cannot deliver a few tablets of sulphonamide for the cure?

These diseases vanished from the West before the advent of vaccines or drugs. Have we not enough vaccines and drugs today to vanquish them? Their non-delivery is the result of the factors responsible for the poverty, and the newer vaccines and drugs are likely to meet the same fate.

The eagerness for the new recombinant technology shown by our scientists is commendable. But experience shows that this penchant for the ‘latest’ Western science and technology and ‘collaborative research’ has caused India to spend large sums on repetitive research that has created dependency and stifled the originality of younger scientists. It has also diverted their attention and Indian resources from the application of available

knowledge and technology which could transform the health and welfare of Indians.

Besides providing a ready-made infrastructure for large-scale trials, the VAP will also provide US scientists with much needed finances for their recombinant research. The Walter Reed Army Hospital, which has shown considerable interest in this programme, will also acquire valuable data and new tools in tropical diseases, an area of major interest.

The recent much-publicized Lentin Commission’s report raises serious doubts as to whether the safety of India’s illiterate poor can be left in the hands of the medical profession, scientists or politicians. The public will have to be made fully aware of the implications of such a programme and must have the final say in the making of decisions as well as the implementation of a study where large numbers of poor people will be subjected to these trials.

Nobody in India will decry the usefulness of vaccines. Unlike family planning, the poor see its utility. Yet it should not divert our attention from the major problem — the need for egalitarian socioeconomic development that alone can eradicate the root cause, namely poverty.

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The October storm

SIR—The Commentary “Modelling the future: a joint venture”, by David Rind *et al.* (*Nature* 334, 483–486; 1988) draws an original parallel between weather forecasting (and climate) models and economic ones. The starting point of the analysis is, on the meteorological side, the inability to predict the exceptional storm which struck parts of Western Europe in October 1987.

Although, it does not affect the conclusion of the paper, this initial statement is wrong. It may have been true in some countries, but we think it important to bring to the attention of your readers that, as acknowledged by the British committee of enquiry, the storm was indeed well predicted by the French weather service. Warnings of exceptionally strong winds (gusts in excess of 42 metres per second) were issued to all relevant users more than 36 hours in advance. As a result, not a single life was lost at sea and human casualties were kept to a minimum over land, in France.

No magic was used in the forecasting procedure.

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More on Benveniste’s dilution results”

SIR—I feel that the experiments of Benveniste and his colleagues (*Nature* 333, 816–818; 1988) can be simply explained by the fact that Fab’ and F(ab’), fragments of antibodies were formed in these serial dilutions of anti-IgE antiserum involving vigorous agitation when the dilutions were prepared. It is comparable to small branches of pine trees being shaken vigorously until the pine-needles fall off. Pine needles can go through a meshwork of a screen, and these antibody fragments specific for human basophils could be carried on the surface of water molecules through serial dilutions.

The authors happened to have chosen one of nature’s most sensitive indicator systems. Heat, freeze-thawing and ultrasound destroyed the antigen specific effect, suggesting even more strongly that we are dealing with a protein or polypeptide substance which would be in keeping with the concept of Fab’ fragments. The authors were right, that they were not dealing with an antibody “molecule”. But we do not have to invent “electronic” or “magnetic fields”, if sub-fragments of antibodies can explain the above phenomena in conventional immunological terms.

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SIR—Upon first perusal, the report of the *Nature* team investigating the work of Benveniste *et al.* seemed reasonable, even fair, notwithstanding the absurdly dramatic touches such as taping the codes to the ceiling. The conclusions left plenty of room for further evaluation and experimentation. However, a glance back at the title of this report was surprising: a *delusion*? This report engendered some uncertainty but did not, to my understanding, expose a delusion. This slap of yellow journalism belies a certain eagerness to mollify an upset scientific community, as well as the much more dangerous intention of the *Nature* team to debunk rather than evaluate. This is further corroborated by James Randi’s letter in *Time* magazine in which he gloats that his erstwhile invalidation of the high dilution study invalidates homoeopathy as well; this betrays monumental prejudice, ignorance and destructiveness. With such bias in evaluating Benveniste’s work, is it not the pot calling the kettle black when they accuse him and his coworkers of a biased attitude and righteously point out that some funding for the study was provided by a homoeopathic pharmacy?

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Taxonomy blooded by cladistic wars

Palaeontologists have buried the hatchet of cladism, to the general enlightenment. But the siege mentality at a meeting last month argues for the preservation of comparative anatomy.

AFTER decades of study of long-dead animals, vertebrate palaeontology in Britain itself now faces extinction, and the situation elsewhere in Europe is not much better. It is ironical that the lives of prehistoric animals are avidly followed in the public prints, but that the academic disciplines from which the scripts of these soap operas spring are being starved. Worse still, comparative anatomy, without which vertebrate palaeontologists would be hardly better equipped to trace the history of life than stamp collectors, may be dragged down as well.

Comparative anatomy does not evoke a natural fondness among most working biologists. To many, the smell of formalin means mustiness more generally. Yet to work on a living organism without sound knowledge of what it is, and how it is put together, diminishes the worth of what results emerge. The tale of the embryologist whose observations of a batch of sea urchin embryos were thought anomalous until it was realized that the species used was itself unusual is not apocryphal.

Even so, the plight of comparative anatomy is at least partly self-induced. The emergence in the 1980s of cladistics, in principle an objective method of building phylogenies from structural similarity between forms, provoked arguments between the newcomers and the traditionalists unusual for their savagery. The result was to corrode the climate in a small community of researchers and to invite outsiders to regard these unseemly wrangles as signs of a subject in terminal crisis.

That is why it matters that the Cladistic Wars are now over. So much was plain at the 36th Symposium of Vertebrate Palaeontology and Comparative Anatomy, at Boulogne last month. Within a single decade, attitudes towards cladistic methods have swung from abhorrence to acceptance; serious taxonomy based on old subjective ways is now almost unthinkable. But even cladistic purists now acknowledge the value of fossils in the resolution of three-taxon problems.

Thus Peter Forey (British Museum, Natural History, otherwise BMNH), once a cladistic hawk, used fossils of the primitive lobe-finned fish *Diabolepis* to resolve a coelacanth-lungfish-tetrapod trichotomy. Coelacanths and lungfish are seen as sister groups, with tetrapods a sister group to both. But the proposed sister-group relationship between lungfish and tetrapods is now less certain. There has

been much soul-searching among the cladists, but the whole of systematics has a surer foundation as a result.

There is room for numerical taxonomy in the new liberalism, as when Garth Underwood (formerly at the City of London Polytechnic) used compatibility analysis to reveal parallelism in the tooth structure of snakes. The view that venom-injecting fangs are the 'crowning glory' of snake evolution seems to be more revealing of people's fear of snakes than of sound taxonomic judgment.

Changes of perspective have also helped with the ordering of sea snakes (Colin McCarthy, BMNH) and modern rock-dwelling lizards (Nick Arnold, BMNH). Each of the 30 or so independently evolved groups of the latter has a parallel solution for life in a difficult environment. But the solutions are substantially determined by ancestry so that, by contrasting environment and ancestry, rock-dwelling lizards can be a test for the extent of convergent evolution.

By mixing comparative anatomy with palaeontology, Jennifer Clack (Cambridge) produced at Boulogne a new answer to the old question of how the earliest land animals could hear. New specimens of *Acanthostega*, an early tetrapod from the Devonian of Greenland, show that while the animal had a stapes, that was too big and well-ossified to have fitted into the otic notch at the back of the skull, traditionally regarded as the rim of a tympanic membrane. Neither did the stapes provide support for the braincase. Instead, it may have controlled ventilation through a spiracle, much as the hyomandibular (the stapedial homologue) does in some fishes. An air pocket in the spiracular cavity would have, incidentally, been an excellent resonating chamber. That is another feather in the cap of comparative anatomy.

Comparative anatomy has also been brought to bear on the question of vertebrate origins by Richard Jefferies and Tony Cripps (BMNH), who demonstrated how vertebrates may have evolved from echinoderm-like animals called calcichordates. Their approach differs from Garstang's 'auricularia' theory of the 1920s in that it is based on real fossils.

At Boulogne-sur-mer, French researchers appropriately led in the study of the earliest, fish-like vertebrates. The taxonomy of Alain Blicek (Lille) was the backdrop for the exciting new faunas from

Vietnam (Philippe Janvier, Paris) and Bolivia (Pierre Gagnier, Paris). The Ordovician ostracoderm *Sacabambaspis janvieri* may hold the clues to the nostril structure of heterostracan ostracoderms (a long-standing mystery) and also illuminate early vertebrate radiation.

Inevitably, the periodic mass-extinction hypothesis of David Raup and Jack Sepkoski is grist to the mill of the new taxonomy. Colin Patterson and Andrew Smith (BMNH) continue to complain that the taxonomic data on which the hypothesis rests are flawed. They say that in Sepkoski's data, drawn from his own compendium of the stratigraphic ranges of marine fossil families and later genera, the naming of fossil species owes more to the circumstances of collecting than biological reality. For example, 68.8 per cent of all fossil fish species are known from unique localities. The figure for echinoderms is 69.1 per cent. But the ecology of fish and echinoderms are very different, so that the figures are too close to be coincidental, which implies that the formal taxonomy of all marine species is based on locality, not biology. And the same may be true for other groups of marine animals.

Patterson and Smith also say that the 'noise' in Raup and Sepkoski's mass-extinction 'signal' from marine families and genera arises from misapplied taxonomy, as when groups treated as monophyletic turned out to be polyphyletic or paraphyletic. Even at the species level, taxonomy has been misapplied to produce names of little more than administrative significance. If this raking over old coals sounds like the cladistic wars at their height, there is a side-benefit: there is a much better idea of the phylogeny of fish and echinoderm genera than before Patterson and Smith's analysis.

But while adversity has removed the luxury of internecine strife, a siege mentality has palpably taken over. Palaeontologists know they have a fustian image among other biologists, but the archaic monographs cited in their papers are a doleful reminder that comparative anatomy has too often been abandoned for less well-founded disciplines, palaeoecology for example. Yet it is not much comfort to know that a 1911 lungfish monograph may still be valid while much work from the 1950s and 1960s will prove of historical interest only: for if comparative anatomy goes, the rest of biology will suffer irreparably.

Henry Gee

Evolutionary biology

Of cuckoo clocks and cowbirds

Paul H. Harvey and Linda Partridge

IN a remarkable paper¹ entitled *The Perfection of Animals* published almost 25 years ago, A. J. Cain concluded that "the grounds on which so much of the diversity of animals has been asserted to be non-adaptive and merely ancestral are mistaken, and based almost entirely on lack of information. The indirect evidence available points strongly to the adaptive nature of the major plans on which animals are built, and of almost all the details". One category of evidence used by Cain in support of his thesis, and by many before and since, is convergent evolution: unrelated animals that invade similar ecological niches often evolve to become similar in morphology and behaviour. The sabre teeth of large carnivores, for example, beautifully adapted for stabbing, have evolved independently in creodonts, machairodont cats and marsupials. Unfortunately, it is still rare for ideas about evolutionary convergence to be amenable to experimentation. Instead, we must make do with the comparative evidence. Brood parasitism in birds provides a welcome exception, as is nicely demonstrated by Brooke and Davies's study of host-egg mimicry in the European cuckoo (*Cuculus canorus*) reported on page 630 of this issue².

Brood parasitism, in which a 'parasite' species habitually lays its eggs in the nest of one or more 'host' species, has evolved on at least seven occasions in different bird families or subfamilies^{3,4}. Europeans are familiar with Old World cuckoos and Americans with cowbirds, but the habit has also evolved on other continents in cuckoos, honeyguides, finches, and in a weaverbird and a duck. Interspecific brood parasitism may have evolved from an intermediate stage of intraspecific brood parasitism^{4,5}. Egg dumping by females in the nests of conspecifics is being found to occur in an increasing number of bird species^{6,7}. Although bird parasites all exploit the parental care of their hosts, they display various lifestyles: there are varying degrees of mimicry of host eggs and nestlings, for example, and young brood parasites may or may not kill their nest mates. Why are there these differences? Do different parasite-host pairs represent different stages in the progression of an evolutionary arms race? If so, what is the denouement?

Stephen Rothstein produced the first extensive experimental study⁸ that tackled such questions. Brown-headed cowbirds (*Molothrus ater*) parasitize many hosts, but unlike the European cuckoo do not lay polymorphic eggs. Rothstein was inter-

ested in whether hosts discriminate against cowbird eggs, so added artificial brown-headed cowbird eggs to nests of potential hosts and found that some host species reject the eggs whereas others do not. Surprisingly, he could find no satisfactory explanation for the difference between the groups: "most or all acceptors and rejectors have eggs easily distinguishable from cowbird eggs, long histories of sympatry with the cowbird, and the physical capability to reject cowbird



Unwelcome guests — the European cuckoo (above) and the brown-headed cowbird (below).

eggs"⁸. Rothstein concluded⁸ that "the host-parasite system of the brown-headed cowbird is not a highly evolved interaction" and that, given time, cowbirds will evolve many cuckoo-like adaptations for parasitizing their hosts⁹. On the basis of a genetic model, he also argued¹⁰ that there are very few species with intermediate rates of rejection because genes causing hosts to reject the eggs of brood parasites would spread through a population in short periods of time, of the order of 20–100 years.

More recent genetic models¹¹ suggest that the rate of spread of rejection genes is likely to be much faster among cowbird than cuckoo hosts, where it would typically take thousands of years, mainly because the proportion of host nests parasitized by cowbirds (20–70 per cent in Rothstein's model¹⁰) is generally much greater than by cuckoos (<1–20 per cent). The models also suggest that the rate of spread of egg mimicry in brood parasites is enhanced by the spread of egg-rejection genes in their

hosts. The models do not explain why cuckoos should have gone on to evolve both host-specific races (gentes) of females and more frequent egg mimicry than have cowbirds. The eggs of some species of cowbird do appear to have evolved in response to host discrimination^{12,13}, which means that egg mimicry can evolve in this group. The models also do not explain why some host species have evolved egg rejection while others have not. There may be an undetected time element involved, as some hosts are more recent than others. But there may also be a considerable amount of stochasticity in the system. Biologists often assume there is tremendous genetic variation because of selection experiments. But such experiments are usually done on a continuous character, such as size, not on more dichotomous characters (accept versus reject), one of which is absent initially (S. Rothstein, personal communication).

Why has the cuckoo evolved host-egg mimicry but not host-chick mimicry? The answer seems to lie not with the cuckoo but with the hosts, who show no signs of resisting cuckoo chicks, despite the fact that cuckoo chicks eject their nest mates and thus reduce markedly the reproductive success of their hosts. Davies and Brooke¹⁴ suggest that the selective forces favouring rejection by the host of cuckoo chicks are not so great as those favouring rejection of eggs: rejection of a cuckoo egg saves the host's current breeding attempt, but by the time a chick is present the brood may be lost. But the rejection of a cuckoo nestling immediately it hatches, and before it has been able to eject the other contents of the nest, would save the brood and may be physically easier to achieve than removing an egg (S. Rothstein, personal communication). Comparison of the reproductive biology of cuckoo hosts with those of hosts whose chicks are mimicked by their brood parasites might be profitable. Chick-replacement experiments could also determine whether the hosts whose chicks are mimicked are more likely to reject exotic nestlings.

Brooke and Davies's demonstration in this issue² that discrimination by hosts is a powerful selective force in the maintenance of both host-egg mimicry and of cuckoo gentes, prompts questions about the evolutionary and population-dynamic consequences of host specialization. The evolution of host-rejection behaviour may be part of the impetus for the evolution of new host preferences by the cuckoo because, among suitable British hosts, those which the cuckoo now favours are less discriminating than those which the cuckoo attacks only rarely¹⁵. One consequence of host specialization may be the increase in parasitization rates of British cuckoos on reed warblers (*Acrocephalus scirpaceus*), a species on which cuckoo reproductive success is high, despite a

Bruce Coleman/Hans Reinhard

Bruce Coleman/Wayne Lankinen

decline in parasitization rates on other species and a probable decline in total cuckoo numbers¹⁵.

How, then, are cuckoo host preferences determined? Direct evidence is lacking, but the best guess is that the appearance and habits of the host species are learned by young cuckoos while they are being reared, so that when female cuckoos come to breed they can subsequently find and recognize the appropriate host species. This type of learning would ensure that host preferences are transmitted from mother to daughter. It is not clear how the same can be ensured for egg colour, because individual males may well mate with females from more than one gens. Such a mating pattern would produce gene flow between gentes, which could disrupt the differentiation in egg colour as well as prevent speciation. In birds, females are the heterogametic sex, and the problem would be solved if the genes determining egg colour were on the Z chromosome, which is passed only from mothers to daughters. Investigating the genetics of cuckoo-egg colour is not a trivial task, because cuckoos and hosts must be bred together. When chick mimicry is involved, males as well as females must carry the genetic material that adapts them to particular hosts. In such cases, host-specific brood-parasite speciation is more likely to follow as assortative mating would be strongly favoured so that both male and female nestlings would mimic their hosts (S. Rothstein, personal communication).

Brood parasites and their hosts have developed a battery of other adaptations as a consequence of the continuing arms race. For example, like other parasitic cuckoos, but not all brood parasites, European cuckoos' eggs are much smaller than would be predicted on the basis of the adults' body size¹. Cuckoos are much larger than their hosts, and an egg of the 'correct' size for a cuckoo would be vulnerable to detection by the host. Indeed, large model eggs are more likely to be ejected by reed warblers¹⁴. Further-

more, larger eggs would probably take longer to hatch, thus giving the host nestlings time to grow large enough to make ejection difficult or impossible.

Female cuckoos are nest predators as well as brood parasites, and they are suspected of being responsible for the destruction of reed warbler nests¹⁴. Seemingly paradoxical, this behaviour makes evolutionary sense if a cuckoo destroys only those nests that are located in its territory after host incubation has started — it would then be too late for the cuckoo to lay in the nests and, by destroying them, she may induce the hosts to produce later broods which she can parasitize. In support of such an explanation is the finding that nests containing cuckoo eggs are less liable to predation. Such behaviour underscores the effect that brood parasites can have on the population dynamics of their prey. To understand which host species are likely to be in danger of extirpation as a result of brood parasitism, more demographic data for

host species are needed. A preliminary analysis¹⁶ points to the central importance of the intensity of parasitism, juvenile and adult mortality rates, number of nesting attempts per year, and the number of offspring fledged by parasitized and unparasitized females. Not surprisingly, hosts of cuckoos and cowbirds direct strong aggression against adult brood parasites that are detected near their nests.

We do not yet know the extent to which the adaptations of cuckoos, cowbirds, other brood parasites and their various hosts represent different snapshots in time of a repeated evolutionary clock. But the potential is now clear for a profitable melding of comparative studies, experiments and mathematical models to analyse the arms races between brood parasites and their hosts. □

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Planetary evolution

Greenhouses and magma oceans

David J. Stevenson

THE present drought in North America has been attributed by some to the greenhouse effect of increased atmospheric CO₂, but a recent minor flood of papers address the question of a primordial water-vapour greenhouse, its implications for the differences between Earth and Venus and whether the Earth was once mostly or completely molten. Jim Kasting of NASA Ames, both independently¹ and jointly with his colleagues² now clarifies some of the important issues in this contentious area, some of which date back 20 years to the original idea of Ingersoll³.

Much of the recent activity has been motivated by Matsui and Abe⁴ at the University of Tokyo. The two questions posed are these: if a water-bearing planet is heated sufficiently (by the Sun or by impacts), will all the surface water evaporate to form a dense atmosphere of steam and, if so, is the subsequent rise in planetary surface temperature so great that the surface rocks melt, causing a global magma ocean? The latter issue is interesting because of the important implications it has for the chemistry in the earliest part of planetary history. Not surprisingly, the answer to both questions is a conditional affirmative; the debate centres around the quantitative criteria that must be satisfied.

The idea behind such a 'runaway' greenhouse is simple enough. Imagine a planet with liquid water at its surface and an overlying atmosphere only of water vapour. Suppose that the outgoing heat

flux that must be transported from the surface to space is gradually increased. As the surface temperature increases, the vapour pressure increases so the atmosphere becomes more dense and opaque. As a result, the surface temperature must increase more rapidly than the effective temperature, which characterizes the infrared photons escaping to space. At some critical heat flow, the effective temperature is determined by the temperature of the uppermost saturated column of water vapour of unit optical depth (over which the light intensity is reduced to 1/e); this temperature is almost independent of the surface temperature. This is the runaway: the planet can not increase its outgoing flux by increasing its surface temperature, and the water ocean completely vaporizes. Any further increase in outgoing heat flow can be accommodated by stabilizing the surface temperature at a very high value, typically about 1,500 K or more for an atmosphere of water vapour equivalent to an ocean on Earth, for which the water vapour becomes partially transparent to near-infrared photons and only achieves saturation very high in an optically thin part of the atmosphere.

Kasting and colleagues address^{1,2} the technical issues concerning the state of this atmosphere — whether heat transport is convective or radiative, the effect of clouds and so on — and conclude that the critical heat flow for the onset of a runaway is around 1.4 solar constants (where 1 represents the present solar input at

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Earth). Without heating by impacting bolides, early Venus would have been only marginally at this value, because the Sun was then less luminous than now, and the Earth would be safely in the low-temperature state both then and now. But what of the impact heating? The gravitational energy released during the creation of Earth in 10^8 years corresponds to almost the equivalent of 1 solar constant. If all or most of this energy was reradiated and if the Earth and Venus were made in this time or less, as generally believed⁵, then a runaway greenhouse effect would seem inevitable for both planets; the fact that Venus is closer to the Sun might then affect its later evolution but would be largely irrelevant at the earliest times.

A consensus seems to be emerging, then, of an earliest Earth and Venus both swathed in steam atmospheres with global magma oceans, conceivably extending to great depth. This defines a wonderful playground for assessing planetary volatile reservoirs and their partitioning between atmosphere and ocean⁶. But are the assumptions leading to this apparent consensus correct? Consider first the assumption that there was a significant content of water in the accreting bodies. It is unlikely that water condensed, even as water of hydration in minerals, at the Earth-forming orbit in the solar nebula, and it must therefore be delivered from more distant regions (the orbit of Mars, perhaps, or beyond).

This does not pose a dynamical difficulty⁵, but it is possible that most of this material was delivered late in the accretion process, and that most of the accretion was 'dry'. Under these circumstances, the Earth was not blanketed in steam and could eliminate the heat of accretion from a surface up to a tenth of which comprised lava lakes which radiate at an apparent (chill crust) temperature about 500 K (ref. 7). No global magma ocean would be needed, either at the surface or immediately beneath the surface. Dry conditions could also arise if planetesimals bearing metallic iron mix with planetesimals bearing water. If this mixing occurs at depth, then the water is almost completely assimilated; both the oxygen and hydrogen enter the growing core of the Earth⁸. Part of the present water reservoir of Earth could have been supplied later (perhaps even around 4,000 million years ago), possibly by comets⁹.

But the most important issue may be the nature of the accretion process. Giant impacts are in vogue^{5,10} (see last week's News and Views article by Glen R. Stewart¹¹) and their consequences cannot be approximated by the uniform, background heat flow used in existing published studies. After one large impact, global melting and a silicate-vapour atmosphere result, but much of the impact energy can

be lost at high temperatures in a time short compared to the time that elapses before the next, comparable impact. Under these circumstances, the typical state of the accreting Earth (the state at all times except for a short period after each impact) could be a chill crust with overlying hot, but mostly liquid, water.

From the perspective of a geochemist seeking to understand differentiation and partitioning inside the Earth, this may not make so much difference, because deep-seated global melting seems unavoidable one way or the other. The partitioning of elements between upper and lower mantle¹² must be elucidated with this in mind. The distribution of volatile constituents is a more difficult problem if there is a complicated chill zone between the magma ocean and the atmosphere most of the time. These challenging issues await further analysis.

Meanwhile, the long-standing issue of why Venus and Earth are so different remains unresolved. The inferred high deuterium-to-hydrogen ratio in the Venus atmosphere could be a steady-state effect of cometary impacts¹³ rather than evidence of a primordial water supply.

Certainly, the work of Kasting and others indicates that if the current Earth were moved towards the Sun by a modest distance, then runaway would occur and the Earth would end up somewhat like Venus. But the history of Venus could depend in part on the stochastic effects of early, very large impacts (or their absence) as suggested by Cameron¹⁴, and not so directly on its distance from the Sun. □

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Atomic physics

Hot favourites for atom cooling

Richard Thompson

LASER cooling is now a well established technique for reducing the kinetic energy of free atoms and stored ions^{1,2}. But until recently it was always limited by the radiative widths of the spectral transitions used to cool the species, to give final temperatures of the order of 1 mK. Now it seems possible to push beyond this limit to attain temperatures in the microkelvin range for clouds of free atoms^{3,4}. Furthermore, complementary experiments^{5,6} with spin-polarized hydrogen atoms are now producing high-density cold clouds of this important elementary system.

Is atom cooling of purely academic interest? After all, just a small perturbation will totally destroy the low temperatures achieved. For sodium, a fall of 1 cm due to gravity raises the temperature by 200 μ K. There are several applications, especially in ultra-high-resolution spectroscopy and the setting of frequency standards. Cooling eliminates the Doppler effect to all orders and permits measurements over very long interaction times (studies of millisecond pulsars are at present limited by the quality of the best atomic clocks). Furthermore, many interesting collision processes can be studied with low-velocity beams and it may not be long before macroscopic quantum phenomena such as Bose-Einstein condensation are observed in the laboratory.

Conventional laser cooling works by using the momentum carried by photons. An atom moving towards a laser tuned to an atomic resonance slows down by a small amount — 3 cm s⁻¹ for sodium atoms — each time it absorbs a photon. To be exactly in resonance in the atom's moving frame, the laser has to be detuned to lower frequencies because of the Doppler effect: this energy defect of the absorbed photon is made up by the loss of kinetic energy of the atom. Spontaneous emission provides a lower limit on the temperature which can be attained. For ideal detuning, this is given by $kT_{\min} = \hbar\gamma/2$ where γ is the natural width of the transition, k is Boltzmann's and $\hbar$ Planck's constant. This gives the value $T_{\min} = 240 \mu$ K for sodium atoms. Now, however, Lett *et al.* report³ a minimum temperature of $43 \pm 20 \mu$ K, a factor of 6 lower. They use a configuration known as optical molasses, where free atoms are irradiated with three mutually orthogonal pairs of counter-propagating laser beams. These apply a viscous drag to atoms moving in any direction. Thus, a region (of typical dimension 1 cm) can be filled with a 'gas' of 10^6 – 10^7 cold atoms performing a random walk which will eventually take them out of the laser overlap region after a period of about 1 s.

One of the four ways by which the

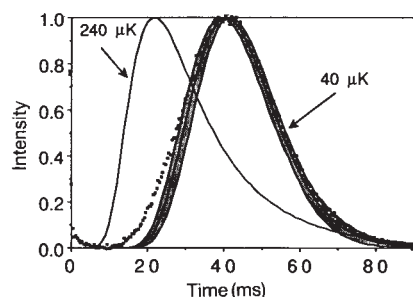


Fig. 1 Time of flight for cooled sodium atoms to travel to the probe region after the optical molasses were turned off in the experiment of Lett *et al.*³ (dots). The calculated curve for 240 μK (left) clearly disagrees with the data, whereas that for 40 μK (right, shading represents geometrical uncertainties) agrees. (From ref. 3.)

temperature of these atoms was measured is a time-of-flight method. The six molasses beams are turned off rapidly and the time taken for atoms subsequently to reach a further beam 1 cm or so below the molasses is measured. As can be seen in Fig. 1, the temperature is about 40 μK , not the expected 240 μK .

At a recent meeting*, C. Cohen-Tannoudji and J. Dalibard (Ecole Normale Supérieure, Paris) and S. Chu (Stanford University) pointed out that the polarization direction in the standing-wave pattern of the counter-propagating lasers is a function of position. The light produces a shift of the ground-state Zeeman levels (atomic sublevels distinguished by their angular momentum component M_z) depending on the value of M_z referred to the local polarization direction. After absorbing and re-emitting several photons, the atoms become optically pumped into the $M_z = 0$ state, which has the lowest energy.

The motion through the polarization gradient then leads to non-adiabatic (discontinuous) transitions to higher-energy M_z states. The energy is extracted from the motion in this process and the atoms are recycled by optical pumping. Thus, cooling occurs as kinetic energy is converted to potential energy and then dissipated into the field. Analysis shows that the minimum temperature achieved by this new mechanism can approach that given by the one-photon recoil energy (2.3 μK for sodium). Other predictions also seem to be confirmed in the most recent experiments.

A second development, from A. Aspect *et al.*⁴, concerns the transverse cooling of an atomic beam by velocity-selective coherent population trapping. Here, the observed temperature is even lower than the recoil energy, but as yet the technique has been used in one dimension only.

The transition used is between two excited levels of helium (the 3S_1 and 3P_1 states) in which the atom has a total

angular momentum (spin plus orbital) $J = 1$. The lower of these, the 3S_1 , is metastable with respect to the helium ground state which has $J = 0$ and cannot be used with this method. A beam of these metastable atoms is irradiated at right angles by two counter-propagating, circularly polarized laser beams that have the opposite sense of polarization (σ_+ and σ_-). The technique works because atoms moving transversely across the beam can absorb and emit photons from the laser, randomly changing their transverse momentum until accidentally having no transverse momentum. Once in this state, however, the atoms are unable to absorb or emit further photons, and hence become trapped in that state.

For a clearer understanding, the internal and translational quantum numbers must be considered. The laser field mixes together the lower 3S_1 states with $M = \pm 1$ via the upper 3P_1 state with $M = 0$ to form two new states, one of which

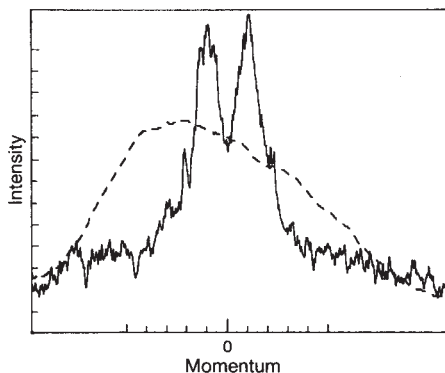


Fig. 2 Transverse atomic momentum profile of a metastable helium beam cooled by population trapping, at the end of the laser interaction region, with the laser on (solid line) and off (dashed line; this profile has been smoothed). The double peak structure at about $\pm \hbar k$ and above the initial distribution is a clear signature of the cooling effect (From ref. 4.)

is absorbing (and emitting) and the other of which is not. For one special case only, in which the linear momentum of the atoms in the 3S_1 state equals that of the photons ($\pm \hbar k$), the two new states have equal energy and are hence 'pure' states. Once in the non-absorbing pure state, the atoms are trapped.

In all other cases, the energy of the new states differs and the atoms oscillate between the two new states with a frequency proportional to the difference in energy. Thus when close to the pure state, atoms can remain non-absorbing for long periods but when far from it they can absorb and emit photons rapidly, exchanging momentum with the field in the process.

A feature of this process is that the atomic beam emerging from the cooling region should have a double peak in the transverse momentum distribution, corresponding to the $M = \pm 1$ sublevels

coupled together. The separation of the peaks is $2\hbar k$ and the width of the peaks gives an indication of the transverse temperature.

Aspect *et al.* irradiated the beam with light at a wavelength of 1.083 μm . Stray magnetic fields have to be reduced to less than 1 milligauss. The observed peak separation is 18 cm s^{-1} (Fig. 2) as expected and the measured half-width corresponds to a temperature of 2 μK , less than the recoil limit for helium of 4 μK . It should be possible to reduce the temperature even further, and the generalization of this method to two or even three dimensions is being considered.

Microkelvin temperatures have also been achieved for a single trapped ion of mercury (J. Bergquist, National Bureau of Standards, Boulder). The ion is confined in a radio frequency ion trap and is first cooled to 1.7 mK by laser cooling on the resonance line at 194 nm (ref. 7). The cooling is continued using a second laser tuned to a weak transition at 281.5 nm. The natural width is very low, so the temperature drops substantially. In fact, the ion is so cold it spends 95 per cent of the time in the ground vibrational level of the trapping potential. The corresponding temperature is around 50 μK .

Progress has also been made in the cooling and trapping of hydrogen atoms at high density. van Roijen *et al.* have achieved a density of $3 \times 10^{14} \text{ cm}^{-3}$ at a temperature of 100 mK and studied relaxation processes in the gas⁵. The spin-polarized hydrogen atoms ($\text{H}\uparrow$) are attracted to a region of low magnetic field produced by suitable coils. They approach thermal equilibrium with the liquid-helium-covered walls, allowing temperature control. To approach the elusive 'Bose-Einstein' condensation that results in all atoms being in the same state, the temperature will have to be reduced by three orders of magnitude at this density: as a next step the authors propose to use optical cooling using hydrogen Lyman- α radiation.

Masuhara *et al.* report⁶ progress using the method of evaporative cooling. A similar $\text{H}\uparrow$ trap is loaded and then the trap depth is reduced, with the $\text{H}\uparrow$ decoupled from the walls. This cools the $\text{H}\uparrow$ by letting the most energetic atoms escape. Masuhara *et al.* achieved a temperature as low as 3 mK in this way, the cooled gas having a lifetime of several minutes. □

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* The 4th Symposium on Frequency Standards and Metrology, University of Ancona, Italy, 5-9 September 1988.

Muscle contraction

Invisible actin makes its debut

John Squire

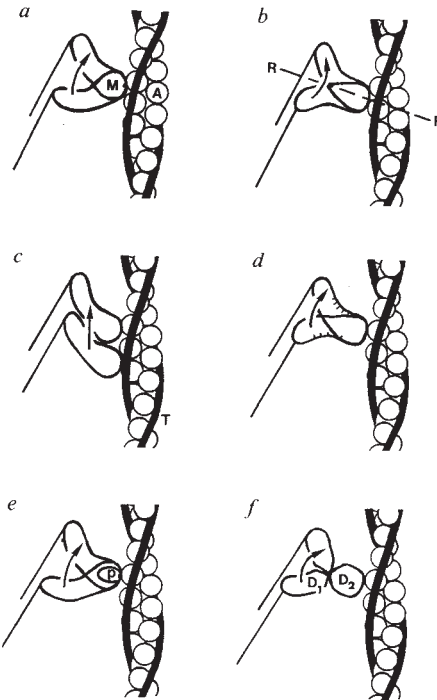
IN attempts to solve the question of what produces the force that makes myosin and actin filaments tend to slide past each other during muscle contraction (see figure), several approaches to determine the structure of these proteins have been used but none has yet come up with a clear answer¹. A paper by Paul Curmi, James Spudich, Robert Mendelson and collaborators in the current issue² of the *Journal of Molecular Biology* describes a novel approach to this problem. These authors have made use of neutron scattering from solutions containing 'invisible' (deuterated) actin. This approach has already provided interesting results on the shape of myosin heads in their different orientations with respect to the actin filament, and it promises to clarify many unresolved aspects of muscular contraction. Apart from the particular result obtained by Curmi *et al.*, their general approach of using deuterated proteins in multicomponent systems has enormous potential to reveal valuable structural information in both muscle filaments and other cell components.

We still do not know whether myosin heads really do swing on actin to produce movement, which is the 'classical' view of how muscle works³, or whether, for example, a mechanism such as that devised by Harrington⁴, in which part of the myosin rod melts, is the main origin of muscular force. The fascinating model systems of Spudich⁵ and of Yanagida⁶, discussed recently in *News and Views*^{7,8}, are designed to reveal how much of the myosin molecule is necessary to interact with actin to produce a viable contractile system. The most recent results of these two groups show that the myosin-head region of the molecule alone, without any part of the myosin rod, is sufficient to support actin filament motion.

Even though the force on an individual actin filament can now be measured, as in the elegant work of Yanagida and colleagues^{8,9}, until it can be shown reliably that exactly the same force per myosin head can be produced in the model systems as in intact muscle fibres, it remains a possibility that the Harrington model is correct. It may be that heads do 'swing' on actin filaments but that this process triggers the melting of part of the myosin rod, and it is this melting which is the chief source of force production. But even if the model systems are able to prove conclusively that myosin heads alone can interact with actin to generate the required level of force, this kind of approach will not reveal the nature of the structural changes that take place

during contraction.

The technique of scattering of neutrons by proteins in solution has the potential to yield not only the radius of gyration of these proteins through the well-known Guinier plot but also, using higher-angle scattering data, information about the shape and structure of the proteins⁹. Curmi *et al.*² use this method to compare the structures of myosin heads free in solution in the presence of magnesium pyrophosphate (Mg.PPi) and at low ionic strength (chemical conditions that are thought to mimic the initial state of head attachment to actin in muscle fibres), with heads bound to actin in the 'rigor' conformation, which is probably similar to a



Schematic illustrations of different ways of producing an axial shift of 4–12 nm of the rod end of the myosin head (M) on actin (A) during the contractile cycle of muscle. The black helices (T) represent tropomyosin strands. *a*, Swing about the actin/myosin interface. *b*, Roll of the myosin head (roll axis indicated by RR). *c*, Simple axial translation. *d*, Distributed shear along the myosin head¹³. *e*, Swing, but leaving a small head domain (p) unchanged and *f*, pivoting of one major domain of the head (D₁) relative to another major domain (D₂)¹³. The new neutron-scattering data² appear to preclude models *d* and *f* unless by coincidence the initial and final states happen to have the same scattering profiles. Model *c* is precluded if there are two structurally distinguishable attached states of the heads on actin¹². Models *a* and *e* remain plausible, with *e* possibly accounting for data from probes on the myosin head¹⁴; *b* remains plausible if, perhaps, unlikely.

transient state at the end of the attachment phase of the contractile cycle.

Neutron-scattering studies of solutions of free myosin heads (actually the proteolytic fragment myosin subfragment 1 or S1) is, in principle, relatively easy to carry out. But elucidating the structure of S1 bound to actin is complicated by the presence of the actin filaments which also, of course, scatter neutrons. Curmi *et al.* overcome this difficulty by preparing actin from the slime mould *Dictyostelium discoideum* which had been fed deuterated *Escherichia coli*. The resulting deuterated actin monomers behave normally and produce normal actin filaments. The neutron-scattering experiments could therefore be carried out on deuterated actin filaments labelled with protonated S1. Because deuterium (D) and hydrogen have very different neutron-scattering powers, the scattering contrast between the solvent and either protonated or deuterated proteins is very different. By choosing a solvent that contains an appropriate mixture of D₂O and H₂O, it is even possible to reduce the contrast of one component, in this case the actin filaments, to zero; this component essentially becomes 'invisible' in the neutron-scattering experiments.

Curmi *et al.* next use their 'invisible' actin labelled with S1 (at a low stoichiometry to reduce inter-head interference effects that they otherwise observe) to compare the neutron-scattering profiles of these heads with the corresponding profiles from S1.Mg.PPi free in solution (also in the presence of invisible actin). And the result? There is remarkably little difference; the radius of gyration of the S1 changes by less than 1.5 per cent and the high-angle profiles are not significantly different out to a resolution of 2.5 nm. In short, the structures of myosin heads free in solution and bound to actin in the rigor conformation are essentially the same.

Curmi *et al.* conclude that, if myosin heads do swing on actin, and if the two states that they have studied genuinely represent the beginning and end of the muscle's 'power stroke', then the heads do not change their shape between the initial and final states but swing essentially as rigid bodies. If any deformation of head shape does occur it must, at most, involve only

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about 20 per cent of the mass of each head. This result confirms almost all previous studies on the shape of myosin heads; no well-substantiated gross difference in head shape has ever been observed in any two different biochemical states of the head, such as in the presence and absence of ATP¹⁰, the molecule which is hydrolysed by myosin to produce force. Perhaps all these data are trying to tell us something that many have been reluctant to accept.

To put these conclusions into perspective, the figure illustrates some of the changes in head configuration on actin that would produce an axial displacement of the 'neck' end of the head of between 4 and 10 nm, as required from studies of muscle mechanics¹¹, and it indicates how

some of these (*d* and *f*) can probably be excluded by the neutron-scattering data. Other evidence is now accumulating¹² that the initial and final stages of the attachment phase of myosin heads in the contractile cycle do represent two distinct attitudes of myosin heads on actin. With the new neutron-scattering data, this result imposes severe constraints on the structural events that might be involved in the contractile cycle. For example, models *c*, *d* and *f* in the figure would all be excluded. □

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AIDS

Novel HIV systems

Robin A. Weiss

IN VIVO models are much needed for AIDS research to test potential antiviral drugs and vaccines. Besides man, only chimpanzees and gibbons have been found to be susceptible to infection with human immunodeficiency virus type 1 (HIV-1). These apes are in extremely short supply in captivity, and are rightly classified as protected or endangered in the wild. The second AIDS virus, HIV-2, has been reported to infect macaques such as the rhesus monkey. It is not yet clear whether any of these non-human primate species, or any other animals, are susceptible to HIV-induced AIDS. The paper by G. Jay and colleagues on page 606 of this issue¹ is the latest of several new reports which use various animal models of the disease.

The HIV-related simian immunodeficiency viruses (SIV) have been isolated from Old World monkeys such as macaque, sooty mangabey, African green monkey and mandrill². Serological evidence indicates that SIV naturally infects African green monkeys, whereas it has not been found in wild-caught macaques in Asia and appears to be restricted to captive primate colonies. SIV infection of African green monkeys and mangabeys is apparently asymptomatic, but macaques develop AIDS, as indeed they do after inoculation with the mangabey virus (SIV_{SMM}). The infection and disease caused by SIV in macaques promises to be of great importance for understanding pathogenesis and for the development of therapeutic agents and vaccines that may be applicable to human AIDS. Nonetheless, animal models of HIV infection itself would benefit the fight against AIDS.

Several promising leads have recently been published to indicate that research on HIV might usefully be conducted with

lagomorphs and rodents. T. J. Kindt's group recently reported³ that two T-cell lines and one macrophage line of rabbit cells transformed by oncogenic viruses can be infected *in vitro* with HIV-1. O.E. Varnier's group⁴ has now shown that rabbits inoculated with HIV-1 or virus-producing cells develop persistent infections. This results in seroconversion and the ability to rescue virus from rabbit leukocyte cultures up to 7 months after HIV-1 inoculation. Rabbits were first used for human retrovirus research by I. Miyoshi's group⁵, which showed that human T-cell lymphotropic virus (HTLV-1) infection readily occurs and is transmitted from dam to offspring via the milk. There is no evidence that rabbits succumb to HTLV or HIV disease. The rabbit/HTLV-1 system, however, has been successfully used for testing vaccinia-based HTLV-1 recombinant envelope vaccines⁶, and similar usage now appears possible for HIV.

Another approach to devising animal systems is to manipulate mice to become susceptible to HIV infection. The generation of transgenic mice carrying the human CD4 receptor gene has been much discussed, but currently seems unpromising as murine cells expressing human CD4 in culture are refractory to HIV infection and replication⁷. Two groups now show that mice with severe combined immune deficiency (SCID, an inherited immunodeficiency syndrome) can be engrafted with human haematopoietic cells to reconstitute human T- and B-lymphocytes. Because SCID mice are immunodeficient, they accept the human cells as a xenograft.

D. E. Mosier *et al.*⁸ inoculated human peripheral blood leukocytes into SCID mice and obtained mice that give a specific human immunoglobulin response to challenge with tetanus toxoid, an antigen to

which the human donors had been previously immunized. Interestingly, human cell lymphomas also appear in the engrafted SCID mice and these are positive for Epstein-Barr virus.

I. L. Weissman's group⁹ inoculated SCID mice with human fetal liver cell suspensions containing haematopoietic stem cells. These cells, and cells from human fetal thymus and lymph node, provide mature T cells and B cells of human origin in the peripheral blood of the mice. The experiments on the reconstitution of SCID mice by human cells open a new horizon for studying *in vivo* haematopoietic cell differentiation and leukaemia. Each animal, however, has to be individually reconstituted. It remains to be seen whether these mice can be infected with HIV, can make human cell immune responses to the virus, or reacquire immunodeficiency.

Recently, two groups at NIH have made transgenic mice carrying all or part of the HIV-1 genome. It has been reported at conferences that M. Martin's group has inserted a full-length, recombinant HIV provirus into mice which were, of course, kept in strict containment facilities. In the paper in this issue¹, Jay's group reports that lesions resembling Kaposi's sarcoma appear in the skins of transgenic mice expressing the *tat* gene of HIV-1. If, indeed, these are endothelial cell lesions that mimic human Kaposi's sarcoma, the result implies that a single viral gene exerts a proliferative effect independent of viral replication and the induction of immunodeficiency.

These findings pose as many questions as explanations. For instance, as HIV genomes are not generally present in human Kaposi's sarcoma cells, does the *tat* protein act to stimulate a paracrine growth factor? And if Kaposi's sarcoma is directly linked to HIV infection, why is this tumour virtually unknown in haemophiliacs with AIDS? Together with the late G. Khoury, Jay had previously reported¹⁰ that mice transgenic with the *tat* gene of HTLV-1 develop peripheral nerve lesions resembling von Recklinghausen's neurofibromatosis. Clearly, there is much to be learned from the action of single viral genes expressed in transgenic animals. □

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Cellular biochemistry

Pseudosubstrates turn off protein kinases

Grahame Hardie

MOST extracellular signal molecules activate target cells by binding to cell surface receptors, and the main mechanism by which binding is transduced into intracellular effects is via modulation of the phosphorylation of target proteins¹. In most cases this is achieved by the receptor activating the production inside the cell of one or more second messengers (such as cyclic AMP, diacylglycerol and Ca^{2+}), which bind to and activate second-messenger-dependent protein kinases. Thus, cyclic AMP activates the cyclic AMP-dependent protein kinase, diacylglycerol activates protein kinase C and, in the presence of Ca^{2+} , the ubiquitous Ca^{2+} -binding protein calmodulin activates at least five protein kinases (phosphorylase kinase, myosin light-chain kinase and calmodulin-dependent protein kinases I, II and III). The activation of second-messenger-dependent protein kinases is thus a crucial event in cellular signal transduction. A recent paper by Pearson, Kemp and co-workers² provides important evidence in favour of a general model explaining how these kinases are switched on and off.

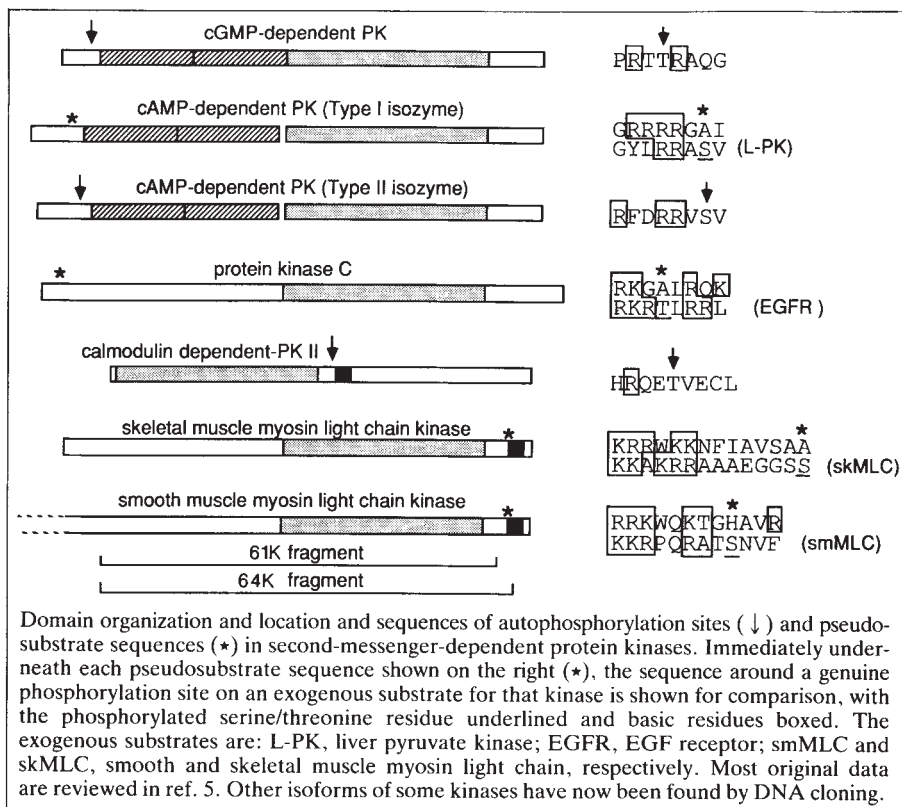
In common with other members of the protein kinase family, the second-messenger-dependent protein kinases contain homologous catalytic domains, coupled with regulatory domains which can either be on separate subunits (as in cyclic AMP-dependent protein kinase) or fused on the same subunit (as in most other cases). For all the kinases shown in the figure, constitutively active catalytic domains can be generated either by dissociation (multi-subunit types) or by limited proteolysis (fused, single-subunit types). Clearly, the regulatory units which are removed by these treatments must contain regions responsible for inhibiting the catalytic domain in the absence of the second messenger. As the substrate for all protein kinases is a small segment of polypeptide, an intriguing idea is that these inhibitory regions work by acting as competitive inhibitors of exogenous substrates.

This hypothesis was first suggested 10 years ago by Corbin for cyclic GMP-dependent protein kinase and the type II isozyme of cyclic AMP-dependent protein kinase^{3,4}. Both these kinases autophosphorylate via an intramolecular reaction, and it was known that arginine residues are critical for recognition of phosphorylation sites by both kinases. Corbin and colleagues found that if the enzymes are treated with the arginine-specific reagent butanedione, autophosphorylation is

abolished and both kinases become constitutively active, although the binding of cyclic nucleotides is unaffected. They proposed that interaction of the catalytic site with the autophosphorylation site normally maintains these enzymes in an inactive state, whereas binding of cyclic nucleotides causes a conformational change which will relieve this inhibitory

serine or threonine in the correct position (asterisks in the figure). Corbin's model can be extended to these cases by postulating that the catalytic sites interact with these 'pseudosubstrate' sequences. In the cyclic nucleotide-dependent protein kinases, the autophosphorylation/pseudosubstrate sequences lie just amino terminal to the cyclic nucleotide binding domain, whereas in the calmodulin-dependent enzymes they are at the amino-terminal end of the calmodulin-binding sequences. It is therefore easy to visualize how binding of cyclic nucleotide or calmodulin could produce a conformational change which disrupts interaction of the catalytic and inhibitory sites.

Several groups have now shown⁵⁻⁹ that



effect. Reaction of butanedione with the critical arginine residues in the autophosphorylation sites also prevents the inhibitory interaction.

Corbin's first model did not explain how the catalytic subunit of the type I isozyme of cyclic AMP-dependent protein kinase is inhibited by the regulatory subunit, as there is no autophosphorylation in this case. For all the kinases shown in the figure, it is known that a particular disposition of neighbouring basic residues is required before a serine or threonine residue becomes a substrate (see ref. 5 for review). As the complete amino-acid sequences of the kinases have gradually emerged over the past few years, it was noticed that some of them, including type I cyclic AMP-dependent protein kinase, have sequences with the right combination of basic residues, but which lack a

synthetic peptides based on the autophosphorylation sites or putative pseudosubstrate sequences in the skeletal and smooth muscle isozymes of myosin light-chain kinase, calmodulin-dependent protein kinase II and protein kinase C are specific competitive inhibitors of their respective kinases, with K_i values around $1 \mu\text{M}$. But this does not prove that the endogenous sequences interact with the catalytic sites in the native enzymes. In their new paper², Pearson *et al.* study two proteolytic fragments of smooth muscle myosin light-chain kinase. A tryptic fragment of relative molecular mass 64,000 (64K) contains all the catalytic domain and the pseudosubstrate sequence (its carboxy terminus is the carboxy-terminal arginine of the pseudosubstrate sequence shown in the figure). This fragment has lost most of the calmodulin-binding site

and is totally inactive in the presence or absence of Ca^{2+} -calmodulin. Further cleavage by trypsin at the carboxy-terminal end of the 64K fragment produces a 61K fragment which is constitutively active. Although the carboxy terminus of the 61K fragment is not precisely defined, the implication is that the pseudosubstrate site has been removed, and this sequence must have been responsible for the lack of catalytic activity of the 64K fragment.

The pseudosubstrate concept offers a potentially fruitful approach to generation of specific protein kinase inhibitors that could be used as experimental tools or, if problems of cell permeability could be overcome, pharmacological agents. Design of such inhibitors is, however, not a simple matter of replacing the serine/threonine in a known phosphorylation site by another residue. This is illustrated by studies of the specific protein inhibitor of cyclic AMP-dependent protein kinase, a thermostable, 11K protein which inhibits the kinase competitively with a K_i of 0.5 nM. Walsh, Kemp and co-workers have shown that a 20-residue peptide derived by V8 proteinase digestion is as potent an inhibitor as the intact protein¹⁰. Intriguingly, the peptide contains at its carboxy-terminal end the pseudosubstrate sequence Arg-X-X-Arg-Arg-X-Ala-X-X-X: this has the same disposition of arginines as

the autophosphorylation site on the regulatory subunit (type II isozyme) of the kinase, but with serine replaced by alanine. Synthetic peptides in which any of the three arginines are replaced by lysine are much less potent inhibitors, confirming the importance of the arginines. But removal of six residues from the amino terminus of the peptide, which leaves the apparent pseudosubstrate sequence intact, also reduces the potency of inhibition 100-fold¹⁰. The design of protein kinase inhibitor peptides which are both specific and potent may require a more subtle understanding of substrate-active site interactions than we have at present. □

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Ocean Drilling Program

A tale of two ridges

*Leg 121 shipboard scientific party**

BROKEN RIDGE and Ninetyeast Ridge, two prominent features in the Indian Ocean, originated along with the Kerguelen Plateau further south, from volcanic activity at the Kerguelen–Ninetyeast hotspot^{1,2} (Fig. 1). New data from this Leg, together with results^{3,4} from Legs 119 and 120, show that the Broken Ridge/Kerguelen–Heard platform was formed more than 90 million years ago (Myr). The Ninetyeast Ridge formed later, during the Late Cretaceous and early Tertiary (up to 40 Myr), as the Indian Plate moved north over the hotspot which was then near a mid-ocean spreading centre^{5,6}. The vol-

ume of lava along the volcanic trail, which is 5,000 km long and 200 km wide, is comparable with that of the Hawaiian–Emperor seamount chain.

The stratigraphy of Broken Ridge reveals clearly the effects of rifting (extension of the lithosphere; Fig. 2). A rifting episode between Broken Ridge and Kerguelen that raised the crest of Broken Ridge above sea level is seen in a distinctive angular relationship (unconformity) between the pre- and post-rift strata. As Broken Ridge submerged again, a thin subhorizontal sedimentary cap was deposited over the truncation surface (Fig. 2). We drilled at four sites (752–755) along a north–south transect to reconstruct the history of vertical motion at Broken Ridge and to elucidate the response of the lithosphere to extension.

The uppermost sediments below the angular unconformity are open-ocean carbonate chalks from the middle Eocene (around 48 Myr) deposited at depths of about 1,000 m. The lowermost sediments above the unconformity are upper Eocene (42 Myr) carbonate oozes containing reworked shallow-water microfossils, coarse sand and gravel. The seafloor

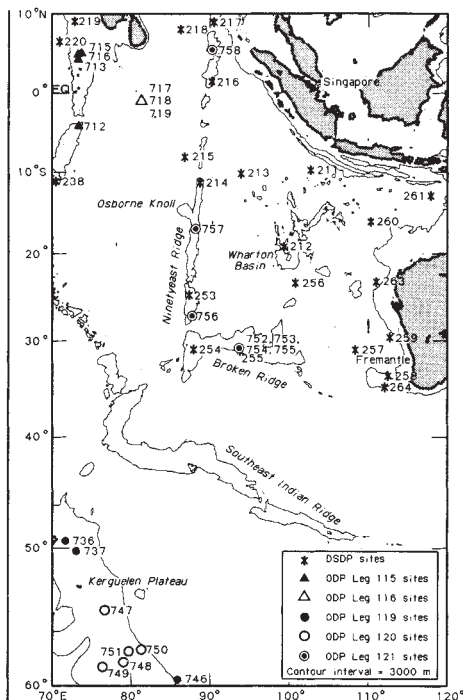


Fig. 1 Location of DSDP and ODP drill sites.

spreading south of Broken Ridge also started in the late Eocene. Thus it seems that rifting started in the middle Eocene, lasted only 3–7.5 Myr and raised the south-facing escarpment of Broken Ridge more than 2 km. Rift flank uplift, as at Broken Ridge, results from flexural isostatic rebound after tectonic unloading of the lithosphere during extension⁷.

The entire dipping, truncated sequence of strata at Broken Ridge was deposited at increasing depths before the rifting. This is consistent with rifting being caused by far-field (intraplate) stress (or passive rifting) rather than by mantle convection (active rifting). The latter would substantially raise the sea floor before rifting occurred. Both the brevity of the rifting event and the low present-day heat flow, 44 mW m⁻², suggest that the cause of the uplift at Broken Ridge was mechanical rather than thermal.

We unexpectedly recovered an expanded section across the Cretaceous/Tertiary (K/T) boundary (66 Myr) at Broken Ridge. Light grey Cretaceous chalks are succeeded by a 6-m thick, dark-green, ash-rich layer, which is in turn overlain by Palaeocene chalks. The diversity of calcareous microfossil species is reduced at the boundary. Hardy 'survivor' forms predominate for several metres into the overlying ash-rich layer until eventually replaced by an assemblage of Tertiary taxa. The ashes were deposited for time more than 1 Myr during which the rate of carbonate deposition was up to eight times lower than in sediments below the boundary and above the ash-rich layer.

The ash-rich layer does not give evidence for the volcanic or impact hypotheses for the mass extinctions that

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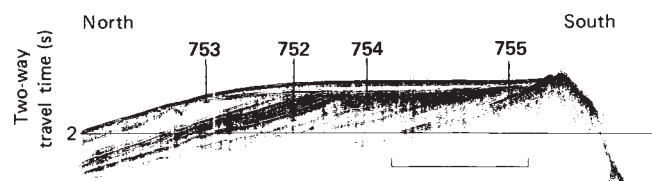


Fig. 2 Seismic line RC2708-20 across Broken Ridge with the positions of the Leg 121 drill sites indicated. (Scale bar, 10 km.)

occurred at the K/T boundary, but is instead an indication of abnormally low levels of planktonic productivity following an ecological crisis at the end of the Cretaceous. The recovery process for calcareous microplankton evidently took longer at the (then) high-latitude location of Broken Ridge than is generally found in low-latitude K/T boundary sections⁸.

Another surprise in the section at Broken Ridge is the large amount of volcanic ash in the dipping and truncated sequence. These basaltic ashes are similar in composition to Kerguelen–Heard Plateau and Ninetyeast Ridge lavas, and we interpret them as recording eruptions at the hotspot to the south-west of Broken Ridge. The amount of ash seems to have decreased from the early Turonian (90 Myr) to the middle Eocene, reflecting either a true waning of volcanism or migration of the volcanic centre away from Broken Ridge. These ashes preserve a clear magnetic-reversal stratigraphy for 75–55 Myr (Chron 33N to 23R).

We sampled the basaltic basement at three widely separated sites along Ninetyeast Ridge (756–758). Basalts from site 756, and also probably from site 757, were erupted above sea level, as found at previous sites on Ninetyeast Ridge^{5,6}. But the lavas recovered at the northernmost site (758) are submarine pillows and sheet flows. Dating of the overlying sediment suggests that the drilled basalts are more than 38, 58 and 80 Myr old at sites 756, 757 and 758. The northward increase in basement age is consistent with Ninetyeast Ridge being the trace on the Indian plate of the Kerguelen/Ninetyeast hotspot⁹.

The basaltic rocks are not primary magmas derived from the mantle, but

moderately evolved tholeiites. Although there are important intra- and inter-site variations in magma composition, ratios of incompatible elements show that Ninetyeast Ridge basalts are similar

to those found on oceanic plateaux, such as Kerguelen–Heard¹⁰. Furthermore, trace-element abundance ratios fall between those of mid-oceanic-ridge and Kerguelen Island basalts (Fig. 3), perhaps indicative of mantle-source mixing before eruption. Such mixing relationships might hold for other trace elements and isotope ratios. Also inter-site compositional variations could reflect long-term changes in the composition of the mantle source.

Microfossils recovered along Ninetyeast Ridge represent a latitudinal transect across different climate zones: temperate, subtropical and tropical. Thus assemblages at site 756, the southernmost site, progress from temperate to subtropical from the Eocene to the middle Miocene (14 Myr), while at site 757, 1,200 km further north, the same progression occurred significantly earlier — upper Palaeocene (55 Myr) to mid-Oligocene (32 Myr). The record at site 758 is temperate in the Campanian (83 Myr) becoming tropical in the upper Maastrichtian (73 Myr). Biostratigraphic timescales, usually based on microfossils from a single climatic zone, are difficult to correlate with those from other zones. The new data will allow inter-zonal relationships to be determined.

The upper 100 m of sediment at Site 758 includes a terrigenous clay component, probably of Himalayan origin, appearing first in the upper Miocene and becoming more abundant later on. This fraction preserves an excellent magnetic-reversal stratigraphy for the past 7 Myr (Brunhes to Chron 6), during which time volcanic ash layers become increasingly abundant. These record eruptions in the Indonesian volcanic arc. Subtle changes in clay content and colour in the topmost sediments reflect relative changes of biogenic and terrigenous input, presumably determined by climatic variations in response to fluctuations in the Earth's orbit. □

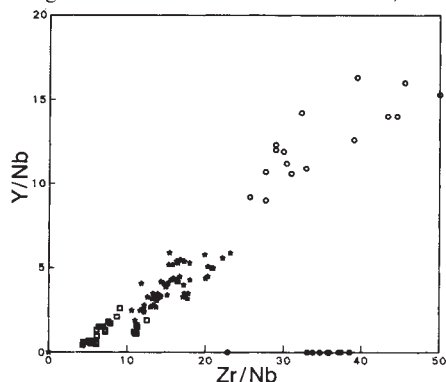


Fig. 3 Zr/Nb-ratio versus Y/Nb-ratio for basalts from Kerguelen Island (□), Ninetyeast Ridge (★) from Leg 121, refs 10–12 and M. Storey and A.D. Saunders (personal communication), and mid-oceanic-ridge basalts (○).

Daedalus

Advanced woolgathering

Wool must be the most inefficiently produced of all agricultural products. Grass takes up a little of the solar energy which hits a field; sheep cropping the grass take up a small fraction of that, and put a still smaller fraction of it into their wool. The energetic efficiency of the whole process can hardly exceed about 10 parts per million. Daedalus is now improving matters by cutting out the middle man. He plans to grow wool directly in a field, much like grass itself.

The biochemistry of the project is simple and elegant, at least in principle. Plants use solar energy to photosynthesize glucose and amino acids, which are the basic raw materials of animal metabolism. And some photosynthetic microorganisms, like microalgae and cyanobacteria, actually release such metabolites into their environment. They can be immobilized in polymeric foam or calcium alginate gel, where they generate these products quite rapidly.

So DREADCO's biochemists are maintaining a section of living sheepskin on a heart-and-lung machine, to discover what nutrients it actually takes up from the blood of the sheep. Glucose is the most obvious and simplest, with various lipids and structural amino acids not far behind. The team will devise a mixed community of microalgae and nitrogen-fixing cyanobacteria, immobilized on a suitable fabric, to supply these biochemicals. The living sheepskin and the artificial photosynthetic surface will then be put together back to back.

The result will be a direct plant-to-wool conversion membrane, DREADCO's Woolfield®. With luck, an element of sheepskin on this nutrient surface will not only flourish and grow wool; it will slowly extend sideways over the fabric, like skin growing to cover a wound. A big sheet of photosynthetic fabric could then be 'seeded' with a distribution of small sheepskin sections, which would gradually enlarge to cover it completely.

Woolfield will simply be laid out to grow, algal side up, wool side underneath. Or it may be supported on trestles like the fabric of an enormous low tent. With a photosynthetic efficiency approaching 1 per cent, a square metre of Woolfield will be as productive as 1,000 square metres of sheep farm, and much less trouble. Thus it could be dyed by spraying colour precursors over it in a suitable pattern, and sheared by simply watering it with a hormonal antagonist to keratin synthesis, to detach the growing wool fibres. Woolfield may even become a useful fabric in its own right, a warm and self-renewing woolly blanket material for tents, roofing and outdoor clothing.

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Existence of syphilis in a Pleistocene bear

SIR—In questioning our claim¹ to have identified signs of treponemal disease in a Pleistocene bear, E. J. Neiberger² seems to be both unwilling to give credit to the power of modern techniques and to be unaware of some published accounts of the disease.

The extensive periosteal reaction and skeletal distribution of lesions in *Arctodus simus* are in fact characteristic of what has been reported in treponemal disease³ and are indistinguishable from diseased treponemal specimens I have examined in medical museums. These differ from the more typically pauci-ostotic, non-spiculated involvement of tuberculosis and other chronic granulomatous diseases⁴.

Spinal involvement in treponemal disease is frequent and is considered appropriate as the site for diagnostic bone biopsy⁵. The non-neuropathic spinal lesions in treponemal disease are characteristically lytic anteriorly, involving two to three adjacent vertebrae with hyperostosis and longitudinal ligament calcification⁶, as in *Arctodus*.

Neiberger's suggestion that such lytic disease (sparing posterior structures) is a "unique finding to tuberculosis" is odd. Malignant processes tend to disregard tissue planes and cause posterior element destruction, not treponemal disease⁷. Nor is posterior destruction produced by invading organisms which, in producing gumma, typically affect the anterior

components⁸.

Neiberger seems to share the popular misconception that treponemal infection has not been found in non-primates, contrary to the evidence from hamsters and rabbits⁹. It is also found in various primates. Determining its presence in other populations would be of value in analysing epidemiological significance. The fact that immunological techniques identified the antigen is not surprising, as antigen survival for more than 100 million years has been documented¹⁰. The presence of antigen only in the walls of the gummas, together with its absence in uninvolved areas of the skeleton or in control materials, makes it unlikely that the antigen is a contaminant from handlers of the specimen.

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Chaos of the Brussels School may not be irreversible

SIR—In addition to the criticism already expressed in the conclusion of Peter Coveney's review article of the Brussels School (*Nature* **333**, 409; 1988) I would like to add another. The essence of this criticism is that all the interesting mathematics of the Brussels school is besides the point, since the school has not explained why the underlying physics should imply any of this interesting mathematics in the first place. In particular, no argument is presented to explain why the underlying (time-symmetric) physics should imply the (overtly time-asymmetric) equations which serve as the starting point for the Brussels school.

It is all very well to talk of systems evolving chaotically into the future, but the real question is why systems cannot then also evolve in the same chaotic fashion into the past (in which case arguments based on chaotic evolution would presumably predict no time variation at all). It is important to note that this problem cannot be resolved by pleading initial conditions — the difficulty lies in getting an asymmetric differential equation in the first place, not in evaluating its consequences.

There are two stringent requirements which must be met if the problem of macroscopic time-asymmetry is to be solved. The first is that the underlying mathematics of the formalism must evolve things backwards in time in the same way that it evolves things forwards in time, because otherwise the analysis is being rigged in advance. Failure to pass this requirement is the shortcoming of the usual thermodynamic 'proof' of the Second Law using Carnot's laws on the maximal efficiency of heat engines. Given that the formalism does evolve things in the same manner in both temporal directions, the second requirement is to show why nature in fact chooses one direction in time over the other. (The trick in passing this second requirement is to do so without invoking an asymmetric argument, thereby dropping you back into the jaws of the first requirement.) Unfortunately, the Brussels school seems to never reach the second requirement because they overtly violate the first requirement.

Trajectories or distributions, single particles or many particles, the underlying physics must still be symmetric. The problem of macroscopic irreversibility is

how to get from this underlying symmetry to an observed asymmetry, not what kind of formalism (chaos and the Brussels school, or coarse graining) is best at expressing such an asymmetry. The Brussels school seems to have simply replaced the asymmetric assumptions Boltzmann was so criticized for using in the derivation of his differential equation (the H-theorem) with a new set of asymmetric assumptions allowing them to derive their differential equation. The shortcoming in the approach is the same: you cannot have an asymmetry serve as the starting point of your analysis of macroscopic physics when there is no such asymmetry in microscopic physics.

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Insertional mutagenesis and breast carcinoma

SIR—Morse *et al.* recently described an interesting case of LINE-1 sequence insertion into the second intron of the *c-myc* oncogene in a human breast carcinoma¹. Repetitive sequences such as the LINE elements are found in multiple copies in the mammalian genome and some of them are capable of transposition².

Several reports have documented the insertion of LINE sequences in various gene loci³. Because the activation of the *c-myc* oncogene may be involved in the pathogenesis of breast neoplasia^{3,4}, it is important to unravel the functional effects of the LINE sequence insertion in the *c-myc* gene in this example. Neither analysis of *c-myc* expression, nor transfection experiments were described in the paper, and the biological significance of *c-myc* insertional mutagenesis is therefore not clear. Morse and collaborators also failed to mention that this was not the first report of LINE sequence insertion in the *c-myc* locus.

We have described LINE insertion 5' to the *c-myc* gene in the canine transmissible venereal tumour^{5,6}. Another example of LINE integration in the *c-myc* locus was recently described in a rat immunocytoma⁷. The discovery of three cases of insertional mutagenesis at the *c-myc* locus by a LINE sequence in various tumours in different species may suggest that these events are not random. The insertion of transposable elements into preferred genome loci is well documented in prokaryotes and similar 'regional specificity' also exists in eukaryotic systems⁸. Some oncogene loci may be preferred integration sites for certain repetitive movable genetic elements and this could result in oncogene deregulation and activation. In the case of the endogenous retroviral-like intra-

cisternal A-particle genome, it was indeed possible to demonstrate that the insertion of this sequence into *c-mos* resulted in its activation^{9,10}.

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DNA and the hydration economy

SIR—The proposal¹ that DNA conformation is determined by “economics in the hydration of phosphate groups” has aroused considerable interest². Central to this hypothesis is the fact that in the A- and Z-forms of DNA the distance between adjacent phosphate groups along the polynucleotide chain is shorter than that in the B-form, so that although a water molecule could form a hydrogen-bonded bridge between successive phosphates in A and Z this is not possible for B. These proposals are based on a survey of the distances between charged phosphate oxygens in adjacent nucleotides and of the location of water oxygens in 13 oligonucleotide structures. The hydration of phosphate groups in the A- and Z-forms is characterized as “economical” in contrast to the situation in B-DNA where the various phosphate groups are said to be “individually hydrated”. This concept of “hydration economy” is proposed as the underlying cause for the B→A and B→Z transitions, both of which occur when the degree of hydration of the DNA is reduced, on the grounds that dehydration will favour those conformations which interact more economically with water molecules.

Saenger *et al.*¹ also consider the effect of salt and organic polar solvents on the conformation adopted by DNA and conclude that “if salt or an organic polar solvent is added, water molecules are withdrawn from the DNA and hydration consequently becomes more economical”. From this argument, salt near the DNA would be expected to favour the

“more economical structures”, that is, A and Z rather than B. It has been known for many years³ that this is not the case for the sodium salt of DNA. X-ray diffractions of fibres⁴ and Raman spectroscopy⁵ show that low levels of salt favour A whereas high levels favour B. It is clear, therefore, that the proposal that “hydration economy” determines DNA conformation cannot be correct.

Saenger *et al.* do not mention the D-form of DNA, whose occurrence for poly[d(A–T)].poly[d(A–T)] has been known for 25 years⁶. We have shown that the transition between the D and B conformations can be followed in time-resolved X-ray fibre-diffraction studies using the SERC Daresbury Laboratory Synchrotron Radiation Source^{7,8}. This transition is induced by varying the hydration of the fibre, with dehydration leading to the assumption of D. The relevance of the D-form stems from its occurrence being favoured by relatively low levels of hydration and relatively high levels of salt⁹. The separation of charged phosphate oxygens along a polynucleotide chain in the D-form¹⁰ is about 7 Å, significantly longer than the 4.4 and 4.8 Å in the Z-form and 5.3 and 5.4 Å in the A-form, and comparable with the 6.6 Å in the B-form quoted by Saenger *et al.* These observations provide further evidence against the ‘hydration economy’ concept, which predicts that phosphate groups in D should, like those in B, be individually hydrated and hence favoured by high levels of hydration. In contradiction to this prediction, our studies demonstrate that the D conformation is a low-humidity form.

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SAENGER *ET AL.* REPLY — Fuller *et al.* doubt our hypothesis¹ because of data² from X-ray fibre diffraction showing that (1) some excess (3–6%) NaCl must be added to DNA to pull well-ordered fibres; (2) with lower concentrations of salt, the A→B transition is inhibited or does not take place even if the relative humidity is above the critical 92%; and (3) the A→B transition occurs above 92% relative humidity when DNA fibres contain ~10% NaCl. Careful interpretation of ref. 4 shows that it does not invalidate but rather provides support for our proposal.

First, at low (< 6%) NaCl content at 75% relative humidity, Na-DNA fibres yield good A-form X-ray patterns because they are highly crystalline. They transform very slowly or not at all into the B-form if the relative humidity is > 92%. We assume that DNA retains the A-form resulting from lattice effects because even at high humidity, crystalline A-DNA

fibres tend to remain in that “metastable” form whereas unoriented (non-crystalline) A-DNA gels reproducibly transform to B-DNA at 92% relative humidity “and remain in that form all the way into solution”⁵.

Second, at concentrations of ~10% in the fibres, excess NaCl crystallizes out⁴, and these samples transform to the B-form at relative humidities > 92%. We conclude that excess NaCl has a destabilizing effect on the Na-DNA crystalline lattice and transformation to B-DNA occurs as observed with A-DNA gels⁵.

Third, solid-state two-dimensional NMR shows that crystalline films (equivalent to fibres) of A-DNA at ~3% NaCl, 75% relative humidity contain up to 60% disordered B-DNA¹¹. This B-DNA is not detected by X-rays, which monitor only the crystalline A-DNA. So, in A-DNA-type fibres, partial transformation to the B-form could have occurred already as it would not be seen by diffraction methods.

Finally, the authors of ref. 4 support our proposal¹ in their last sentence: “. . . the salts . . . cause the fibres at high humidity to take up extra water with consequent dissolution of the A crystallites and formation of the semi-crystalline B form, which is more highly hydrated”. This is the essence of our paper¹ in which we describe why B-DNA is more highly hydrated than A- and Z-DNA. There is a threshold water activity where transitions between highly hydrated B-form and the less hydrated A- and Z-forms occur, no matter whether DNA is in the fibre state or in solution^{12,13}.

We have not considered poly[d(A–T)] because DNAs with special base sequences can behave differently because of the formation of ordered water structures (spine of hydration) in minor and/or major grooves¹⁴. We made clear in our paper that our proposal was for mixed-sequence DNA and for poly[d(G–C)].

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Encoding the Universe

Stephen Muggleton

The Universal Turing Machine: A Half-Century Survey. Edited by Rolf Herken. Oxford University Press: 1988. Pp. 674. £55, \$145.

IN 1937 Alan Turing's description of a general purpose computing machine was published in the *Proceedings of the London Mathematical Society*. Computers based on Turing's ideas are a fact of modern life. But there is still scant awareness of the wide-ranging implications of the thesis behind his work, according to which a simple device with a limited internal memory and a movable, rewritable tape can compute every effectively calculable mathematical function.

At first sight Turing's thesis would appear to be a statement about mathematical functions. Indeed, this is the context in which Turing described his 'universal' machine. A year earlier, in 1936, Alonzo Church gave an account of a mathematical system, called λ -calculus, with which he showed that not all logically well-stated questions can be decided. According to Church, all effective computations could be carried out by manipulating formulae within the λ -calculus. Turing showed that his machine had the same computational power as Church's λ -calculus. The important difference between the two approaches is that whereas Church's λ -calculus represents a universal mathematical notation for calculation, Turing's universal machines form the vital link with physical computability.

Turing's thesis can be viewed as a claim not only about the limitations of mathematics but also about the limitations of physical process. If there were a physical process which could be used to compute a function which a universal Turing machine could not compute, the thesis would be false. If the thesis is true then there is no such process, and universal Turing machines are sufficient for modelling all of physics. To answer quibbles about the task of simulating, say, quantum mechanical components of such a process, David Deutsch (*Proceedings of the Royal Society, London A* 400, 97–117; 1985) has provided an appropriate quantum-based 'fix' to universal Turing machines. Indeed our whole observable universe could be regarded as one huge, loosely coupled computational network. Furthermore, if we accept the physical basis of life processes, we are forced to conclude that computers could, in principle, simulate

any biological function, including the ability to think. Whether such simulation could be realized in practice then has to be reasoned on other grounds.

The wide-ranging consequences of Turing's ideas are reflected in the diversity of subjects dealt with in this book. Apart from being a vital reference work for computer scientists and mathematicians,

The second and main part of the book deals with applications of computation theory within a variety of scientific research areas. Among them is an account by Arbib of self-reproducing machines, an idea originated by von Neumann as a theoretical description of biological processes. These 'cellular automata' were designed not only to reproduce but to contain universal Turing machines for computing how to react to their environment.

A number of contributors discuss the problem of measuring physical complexity. Some physical structures and actions are more complex than others. For instance, imagine giving an adequate description of the structures of both a pure crystal, such as diamond, and a human

body, when each contains an equal number of particles. Clearly, the repeated pattern of diamond allows a far simpler description. Kolmogorov, one of the founders of modern statistics, was the first to suggest that the absolute information content of an encoded description could be defined as being equal to the minimal length of a universal Turing machine program which exactly reproduces the given description. Among the researchers who have further developed this idea, two of the contributors, Chaitin and Bennett, have brought out the close relationship between minimal Turing machine descriptions on the one hand and statistical and physical notions on entropy on the other.

Turing himself was deeply interested in the use of digital

computers for simulating human intelligence. However, not all authors in this volume believe the goals of machine intelligence to be entirely achievable. Notably, Roger Penrose uses Gödel's incompleteness theorem to argue that there may exist some as yet unknown physical laws which allow human beings to exhibit mental abilities which cannot be simulated by machines. Turing gave a simpler explanation. He suggested the possibility of a computer programmed to learn and permitted to make mistakes. In a lecture delivered in 1947 he stated that there "are several theorems which say almost exactly that ... if a machine is expected to be infallible, it cannot also be intelligent.... But these theorems say nothing about how much intelligence may be displayed if a machine makes no pretence at infallibility". These ideas form the basis of the rapidly evolving science of machine learning — according to Michie's article, the missing link in the Japanese Fifth Generation computer project. □

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ON COMPUTABLE NUMBERS, WITH AN APPLICATION TO THE ENTSCHEIDUNGSPROBLEM

By A. M. TURING.

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The "computable" numbers may be described briefly as the real numbers whose expressions as a decimal are calculable by finite means. Although the subject of this paper is ostensibly the computable numbers, it is almost equally easy to define and investigate computable functions of an integral variable or a real or ϵ predicates, and so forth. The fundaments, however, the same in each case, and I have for explicit treatment as involving the latter, shortly to give an account of the relations of the theory of functions of a real variable to the theory of functions of a real variable. According to my definition if its decimal can be written down by a machine.

In §§ 9, 10 I give some arguments with computable numbers include all numbers regarded as computable. In particular, of numbers are computable. They include all algebraic numbers, the real parts of the numbers π , e , etc. The computable numbers, and an example



it also provides an up-to-date source for those interested in research on computation-theoretic models applicable to physics and biology. Among the authors of the survey are Stephen Kleene, Church's student and co-developer of λ -calculus, and Donald Michie, who worked with Turing in the Bletchley Park establishment for decoding messages during the Second World War.

The book is divided into two parts. In the first section Hodges, Kleene, Gandy, Feferman and Davis provide descriptions of the historical and theoretical background to Turing's work. There is some overlap with previous historical accounts, such as Hodges's biography of Turing, and overlap between the contributions themselves — thus we are repeatedly asked to pay heed to Leibniz's injunction, "Let us calculate". Nonetheless, a fair amount of material is new. For instance Davis's account of the development of mathematical logic and its relationship to modern computing should be stimulating for those interested in the history of computing, as Davis himself has played an important role within that history.

Tales of Everyman

Robert Foley

Encyclopedia of Human Evolution and Prehistory. Edited by Ian Tattersall, Eric Delson and John Van Couvering. Garland:1988. Pp.603. \$87.50.

AFTER a holiday spent reading detective stories I have come to appreciate a good plot, with all being revealed in the final chapter. In this context the *Encyclopedia of Human Evolution and Prehistory* is a disappointment. By the time Q is reached all of the mystery has been efficiently cross-referenced out of existence; U and V are decidedly thin, and XYZ a hurried anti-climax. Zuttiyeh (cave site, excavated 1925 and 1926, with hominid fossils of modern affinities ante-dating the neanderthals) is no substitute for the criminally inclined butler. But the let-down is predictable after the cracking start one would expect from a book about Archaeology and Anthropology, in which fossils like *Australopithecus* and *Archaic sapiens* from Africa, Asia and Australia are described in the context of their Adaptations and associated Artefacts.

In virtually every other sense, however, this book is anything but a disappointment. The editors have gathered together some leading palaeoanthropologists and pooled their collective knowledge on all aspects of the subject. To be found here are details on all of the principal sites, the fossil hominid specimens, their archaeology, and discussions of their affinities and morphology. For student, researcher and teacher this will form the most complete source of basic information on the subject. Alongside the main entries (which are often illustrated) are more discursive essays on the concepts and methods employed — classification, culture, phylogeny, preadaptation, for example — as well as summaries of related disciplines. Also included are some short biographical entries, for which the qualification is either death or a pension.

Palaeoanthropology is a notoriously controversial subject characterized by shifting opinions and lack of consensus, and it may be argued that it is inappropriate to give an encyclopaedic authority to what might be nothing more than a passing fashion, or an abbreviated certainty to what is still unresolved. This danger is mitigated by the inclusion of short bibliographical notes, and is easily outweighed by the fact that despite the controversial image of the subject there is a fundamental database about which there is general agreement. Much of this has a muddled terminological history, and the editors are to be congratulated on providing easy access to the raw material of palaeoanthropology. A brief introductory frame-

work is provided, but the lack of a storyline rooted in current interpretations should give this book a longer shelf-life than most others on human evolution.

An incidental benefit of a volume such as this is that it allows us to monitor trends in the subject. One such is the increasing tendency to see palaeolithic archaeology and traditional physical anthropology as a single integrated subject (palaeoanthropology), and there is appropriately extensive coverage of many aspects of the archaeology of the hominids. Another is the emphasis not just on the hominids themselves, but on their environmental context. Against this, phylogeny still takes precedence over ecology, behaviour and function. Cladistics, classification, systematics and phylogeny between them rate nearly 12 columns, and the supremacy of cladistics

is shown by its allocation of over two and a half columns against six lines for numerical taxonomy. Ecology gets just over half a column and natural selection has no entry, while selection is cross-referenced to evolution (eight and a half columns). Surprisingly, punctuational evolution is the preferred mode of evolutionary change; the importance of adaptation is minimized and linked spuriously to gradualism. There is no entry for biomechanics or functional anatomy.

Whatever direction the subject is to take, it will be well served by this comprehensive, clearly written and illustrated guide to human evolution. □

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Off the beaten track

Stuart Sutherland

Eccentrics: The Scientific Investigation. By David Joseph Weeks with Kate Ward. Stirling University Press:1988. Pp.259. £27.50.

ALTHOUGH eccentricity should come naturally, it can to some extent be cultivated. There was an Oxford don who used to hide under an upturned bath in the fountain in Tom quad. When anyone tapped it, he would open it a fraction and snarl "Go away. I'm an oyster". There was perhaps too much method in his madness, but both in this and other ways he fulfilled the mark by which you shall know the genuine eccentric: someone who is odd in an interesting and bizarre way.

Dr David Weeks and Ms Kate Ward claim that *Eccentrics* is the first scientific study of the topic. The book has received considerable praise, so it is worth considering why the word 'scientific' should be deleted from the title.

The authors found their eccentric subjects by placing in pubs, launderettes, supermarkets and other public places an advertisement reading, "Eccentric? If you feel that you might be, please contact Dr David Weeks . . .". The advertisement came to the attention of the media and the authors claim that it was seen or heard by 30 million people of whom 130 submitted themselves for investigation. This procedure ensured that no eccentrics who were unaware that they were eccentric were examined. Of more importance, it must have resulted in a very biased sample because those with the time and inclination to submit themselves to Weeks's investigations cannot have been representative. Even more disastrously, there was no control group, although one could easily have been obtained by an advertise-

ment beginning "Not eccentric? If you think you are not eccentric . . .". The absence of such a control group vitiates the whole study. For example, Weeks claims that his subjects were usually garrulous but this may be a characteristic of people who answer advertisements like his rather than of eccentrics.

Having snared his 'eccentrics', Weeks took case histories and gave them questionnaires to answer. Most of the extracts given from the case histories are unremarkable except for their incoherence. Unfortunately, none of the subjects appears to have met the criteria of eccentricity. The use made of personality tests is, to put it mildly, incautious. Such tests tend to reflect not a person's genuine personality, but the personality he would like to persuade the investigator that he has. Moreover, Weeks was dealing with volunteer subjects, who were presumably determined to persuade him that they were unusual, an endeavour in which they were reasonably successful because their results tended to lie at the extreme ends of the different personality continua.

One could go on. Weeks and Ward seem to be unaware that the very concept of a personality trait is in doubt and that everyone behaves in different ways in different situations. They seem to think that creativity is a unitary trait, although in fact it is restricted in everyone to those areas that he or she has mastered. The book contains a great deal of the gobbledygook beloved of social scientists. Few will want to disagree with the statement that "Most of the time language behaviour involves a dyadic relationship between a speaker and a listener".

The authors might perhaps have done better to spend some time in an Oxford Senior Common Room, even at the risk of their scientific integrity. □

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Life at the top of the tree

R. D. Martin

Comparative Primate Biology. Series editor J. Erwin. Alan R. Liss: 1986–1988. Five volumes*.

THE biennial congresses of the International Primatological Society, the latest of which was held in Brasilia in July 1988, regularly attract over 500 people. Yet the participants tend to cluster into distinct subject areas and there has in the past been relatively little communication between them. Things are changing, however, the stimuli being the urgent need for primate conservation and the growing amount of work on reconstructing phylogenetic relationships. As further evidence that the rolling stones of disciplinary specialization are being pushed back, we now have the appearance of a series of volumes bearing the main title *Comparative Primate Biology*.

The work consists of five volumes, containing 71 chapters and taking over 3,000 pages, and stems from a project conceived in late 1980 and developed under the guidance of J. Erwin, the founding editor of the *American Journal of Primatology*. The goal was to provide a reference collection of original, scholarly reviews, organized within a framework of primate systematics (the series will, in fact, be extended by occasional review articles in the journal). The comparative perspective was chosen not only because of its obvious practical and theoretical importance, but also to combat a regrettable tradition of generalization from a few primates to the whole order. To take just one example, the ovarian cycle of primates is often referred to as the 'menstrual cycle' although lemurs and lorises totally lack menstruation. The comparative perspective also highlights gaps in our knowledge. Moreover, an intriguing point emerges from Dukelow's preface to Vol. 3: many investigators apparently switched to primates other than rhesus monkeys when exports of this species from India were banned; thus, a measure designed to protect rhesus monkeys led to the accumulation of more data on other primate species and hence to an increased need for

comparisons between them.

Comparative Primate Biology is essentially a reference work, primarily suited for libraries. The contents are unlikely to be digested by a single reader; rather, they provide a menu from which dishes can be selected for an occasional meal. Single volumes may well be of interest to particular circles of primatologists, but contributions do not always appear in the expected place — for instance, chapters on seasonality of reproduction and on breeding in zoos appear in Vol. 2B rather than in Vol. 3. A further drawback is that there were obviously delays in the delivery of some contributions and certain chapters (especially in Vols 2B and 4) were manifestly written some years before publication. This is not a fatal flaw, but primatologists seeking up-to-date surveys must look elsewhere.

Volume 1, on systematics, evolution and anatomy, is the biggest of the batch, reflecting the widespread interest in phylogenetic relationships. The chapter by MacPhee and Cartmill on the cranial base merits special mention. It is a well-written, superbly illustrated review of a topic that plays a prominent part in the interpretation of phylogenetic relationships. Other gems are the useful surveys by Tattersall of Malagasy lemurs, by Ford of relationships among New World monkeys, by Covert of early Cenozoic primates, by Kelley and Pilbeam of Miocene large hominoids, and by Nute and Mills on basic principles of macromolecular evolution.

There are, however, signs of over-benevolent editing. The contribution by Ward and Brown on the face of *Sivapithecus indicus*, good though it is, can hardly be called a review. It is also regrettable that Schultz gives only a narrow account of the forelimb in leaf-monkeys, when there is a comprehensive review by Sigmon and Farslow of the hindlimb in primates generally, and the lack of a chapter on chromosomes is a serious omission. On the other hand, purchasers of this volume receive an interesting perk: two disks of gibbon song recordings.

One crucial issue in this volume, which affects the others, is classification. The editors did not impose a uniform system and, as a result, various taxonomic schemes are used. In his preface, Swindler hails this as "a sign of healthy science", but it is better seen as an insidious ailment. Confusion persists in the literature about the overlapping, but nonetheless distinct, undertakings of phylogenetic reconstruction and classification. This has not been helped by the unresolved conflict between classical and cladistic philosophies. Many authors regard the presentation of alternative classificatory schemes as a challenging experimental exercise, but thereby neglect the fact that relatively stable classification is a prerequisite for other

work. It is high time that matters were put to rights — the present situation is comparable to that of a team of computer programmers arguing endlessly about whether they should use Basic, FORTRAN or Pascal, rather than getting on with the actual programming job in hand.

Volume 2 is split into two parts, broadly following the division between field and laboratory studies. The first deals with behaviour, conservation and ecology; given the mounting importance of primate conservation efforts, it is particularly pleasing to see the inclusion of a comprehensive expert review by Mittermeier *et al.* There is an interesting survey by Southwick and Smith of the growth (and recent decline) of primate field studies, and other noteworthy contributions by Coelho on time and energy budgets, by Bernstein and Williams on social organization, by Nadler *et al.* on adult sexual behaviour, by Snowden on vocal communication and by Epplé on olfactory communication.

The second part of Vol. 2, by far the shortest of the set, deals with behaviour, cognition and motivation. In addition to the handy reviews by Lindburg on seasonality of reproduction, and by King and Mitchell on breeding primates in zoos, there are also informative accounts by Meador *et al.* on learning, cognition and intelligence, by Candland on tool use, and by Goosen on social grooming. ▶

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* Volume 1 *Systematics, Evolution, and Anatomy* (Daris R. Swindler and J. Erwin, eds), pp.820, \$190, £167.50; Vol. 2A *Behavior, Conservation, and Ecology* (G. Mitchell and J. Erwin, eds), pp.633, \$150, £132.50; Vol. 2B *Behavior, Cognition, and Motivation* (G. Mitchell and J. Erwin, eds), pp.296, \$100, £88.50; Vol. 3 *Reproduction and Development* (W. Richard Dukelow and J. Erwin, eds), pp.497, \$120, £106; Vol. 4 *Neurosciences* (Horst D. Steklis and J. Erwin, eds), pp.769, \$190, £167.50.

The next volume deals with reproduction and development, and is the most tightly organized of them all. The reviews are arranged in a logical sequence and are relatively even in quality; the standard of illustration is particularly good. In his preface, Dukelow underlines the importance of studies of reproduction for conservation. The special value of the comparative approach is demonstrated most particularly in the contributions by Robinson and Goy on steroid hormones and the ovarian cycle, by Enders and Schlafke on implantation, by King on the morphology of the placenta and fetal membranes, by Brizee and Dunlap on growth (including a useful survey of gestation periods), and by Watts on skeletal development. The latter, incidentally, reveals an important gap in our knowledge: virtually nothing is known about skeletal development in prosimians.

Volume 4 is devoted to the neurosciences, and it too contains a number of good, well-illustrated surveys. It is perhaps unfortunate that, in the preface, Steklis refers to prosimian, monkey, ape and human as constituting a "quasi-evolutionary series", thus perpetuating the misleading notion of the *Scala naturae* as a suitable model for comparative study of primates. The contribution by Stephan *et al.* provides a survey of allometric analyses of the size of brain and parts, from one of the leading research groups involved with this topic. The review by Allman and McGuinness of the visual cortex is simply superb. Other highlights are the competent reviews by Rodieck of the primate retina (which unfortunately has little to say about prosimians), and by Eberhart of neural and hormonal correlates of sexual behaviour.

Despite the unavoidable drawbacks of such a vast undertaking, the overall result is worthwhile. The editors have succeeded in producing a work that should be widely consulted, and the generous triple indexes (taxonomic, author and subject) will greatly facilitate its use. The only previous endeavour of this kind was the multi-volume *Primatologia* (edited by Hofer, Schultz and Starck). Although that work is now well out of date, some of the reviews stand as classics that are still worth consulting. The same will surely be true of some of the contributions in *Comparative Primate Biology* in the years to come. □

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• Also new for primatologists from Alan R. Liss is *Ecology and Behavior of Food-Enhanced Primate Groups*, edited by J. E. Fa and C. H. Southwick. The book is Vol 11 in the Liss series *Monographs in Primatology*, and presents studies of primates whose food is provided for them, contrasting these with the behavioural patterns of wild primate groups. Price is \$59, £52.50.

Complex defences

Fred S. Rosen

The Complement System. Edited by K. Rother and G.O. Till. Springer-Verlag: 1988. Pp.535. DM180, £62, \$98.

THE long dispute between Ehrlich and Bordet, over whether complement is a single substance or a multiplicity of substances, makes for charmingly ingenuous reading today. At a time — the first decade of this century — when the world was still ravaged by tuberculosis, diphtheria and a host of now almost-forgotten infectious diseases, the public's imagination was captivated by the idea that something in the blood could indiscriminately kill germs. Complement, for example, in its English guise as the opsonins of Sir Almroth Wright, gave us that Shavian masterpiece, *The Doctor's Dilemma*.

In the end it turned out to be very complicated. The 20 proteins that constitute complement, and the complex interactions between them and their cell-membrane receptors, now stretch the credulity of medical students and leave most scientists in a state of stunned insouciance. Like the study of the clotting system, the complement system has been relegated to a kind of scholastic pursuit of a few crazed devotees. That is a pity because it has unique and exciting aspects for geneti-

cists, biochemists, cell biologists, immunologists and microbiologists, and in clinical medicine.

Rother and Till have brought together a roster of experts from both sides of the Atlantic, and Japan, to give an account of our considerable knowledge of this subject. The results are very good and would certainly astound, if not embarrass, both Ehrlich and Bordet. During the assembly of the material, however, the genes encoding almost all of the complement components and their known receptors were cloned. This structural information will, one hopes, be included in the next edition of this otherwise valuable tome.

The book contains detailed descriptions of how assays for complement and its components are performed, and how genotyping of the polymorphic components is done (I know of no other single volume where such information is conveniently gathered together). Selective reading of the contents can provide a broad picture of the interactions of the complement proteins and their receptors, as well as their importance in pathophysiology and disease states, but at another level the book also gives the detailed information that one might expect to find in a laboratory manual. □

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Going for gold — Hermes Trismegistus, the father of alchemy or the 'Hermetick Philosophy', here indicating the twin principles of the Work, Sulphur and Mercury (the Sun and Moon). The sphere which Hermes holds is a reminder of the cosmic agent whose influence is necessary for the crystallization of the Philosophers' Stone, the goal of alchemy, which is capable of healing all ills and transmuting all metals into gold. The picture, from Michael Maier's *Symbola aureae mensae* (1617) is reproduced in *The Golden Game: Alchemical Engravings of the Seventeenth Century* by Stanislas Klossowski de Rola, just published by Thames & Hudson, price £30, \$45.

A cosmic book

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Modern theories of the origin of large-scale structure depend on a variety of stimulating new ideas about the physics of the early Universe. In the absence of clean observational tests one can obtain figures of merit for the theories by counting and weighing the plausibility of each of those basic elements that are not substantially supported by observation or mature fundamental theory. A survey of the speculative elements of the leading rival theories illustrates the exciting and highly uncertain state of research in the physics of the early Universe.

WILL our feeble minds ever comprehend the evolution of the Universe? Cosmologists devote their careers to this elusive goal. The large-scale structure of the Universe contains the clues to its origin some 15 thousand million years ago in a primaeval fireball. Subsequently stars, galaxies and great clusters of galaxies were formed. The painstaking process of piecing together the evidence slowly accumulated over decades of observation by astronomers enables the cosmologist to develop theories for the observed large-scale structure. Needless to say, there is no unique viewpoint, and indeed we are far from a complete understanding of the origin or even the nature of what is observed. All of this became very evident during the course of a recent workshop on galaxy formation at Taos, New Mexico, from 4 to 9 January 1988 under the aegis of the Centre for Nonlinear Studies and Institute for Geophysical and Planetary Studies, University of California, and the Los Alamos National Laboratory, which brought together some 50 cosmologists with a diverse array of interests. The proponents of many of the principal theories of large-scale structure were given equal time to advocate their models. The experience stimulated us to prepare a summary of the state of theories of the large-scale structure of the Universe.

There are several rival theories that can be adjusted to account for many features of the observed Universe. Each theory requires assumptions of debatable merit, and confronts diverse types of

observations with varying degrees of success. To evaluate the strengths and weaknesses of these rival theories, we propose a system of assigning odds to the basic elements of the theories. This cosmic book, as we call it, contains the base level of probability, $\mathcal{E}$, to various powers: we loosely interpret $\mathcal{E}^2$ as an appeal to an idea that is highly speculative but not entirely implausible in view of the general observational and theoretical outlook. To each key assumption, then, we assign an *a priori* probability of truth as judged by us; our measure of probability of any given theory being true (in the absence of a clean observational test) is found by multiplying together appropriate probability factors. Our cosmic book is presented in Tables 1 and 2.

We have not made the book on detailed observational tests of the models because the observational situations of the different models have been surveyed in very different detail. Thus the third model in Table 2 seems particularly hard-pressed in part because it has been studied in the most detail. Our odds do reflect the observations to some extent because as the observational net tightens, models can be saved by adjusting assumptions, often at the expense of added powers of $\mathcal{E}$. This adjustment process accounts for the high power of $\mathcal{E}$ for the first model in Table 2. We have yet to discover the minimum power of $\mathcal{E}$ that will discourage the punters.

The basic elements

Let us consider first the density parameter, Ω , defined to be the ratio of the mean mass density to the critical Einstein-deSitter value (for which surfaces of constant world-time have negligible curvature and the cosmological constant Λ is negligibly small.) For a decade or more, observational estimates from dynamics have indicated that $\Omega \approx 0.1$ (ref. 1). The standard scenario of nucleosynthesis in the early Universe, which successfully predicts the pregalactic abundances of the light elements ^2H , ^3He , ^4He and ^7Li , also requires $\Omega \approx 0.1$ (ref. 2). Therefore we assign our top probability, $\mathcal{E}$, to this possibility, as indicated in the first entry of Table 1. Other considerations, based on inflation³ or more subjective criteria of simplicity or elegance of the initial conditions, favour $\Omega = 1$. If Ω is substantially greater than 0.1 then to account for the additional mass density, one must either abandon the standard scenario of light element nucleosynthesis or else invoke some exotic form of non-baryonic dark matter that does not directly participate in the nuclear reactions. Particle physics theories lead one to suspect the existence of massive neutrinos or even more exotic stable dark matter candidates such as the photino or axion. If such particles exist, then it is likely that they interact sufficiently weakly with matter to have survived the hostile environment of the beginning of the Universe and so could provide an important contribution to Ω (ref. 4). For example, if massive neutrinos decoupled from the cosmological heat bath after muon pair annihilation and before elec-

Table 1 A cosmic book: the elements

The cosmological model	Probability
(1) $\Omega \approx 0.1$ and	
(a) zero space curvature	$\mathcal{E}$
(b) $\Lambda = 0$	$\mathcal{E}$
(2) $\Omega = 1$ with dominant mass in	
(a) HDM	$\mathcal{E}^2$
(b) CDM	$\mathcal{E}^3$
(c) baryons	$\mathcal{E}^3$
(3) New physics: failure of the	
inverse square law, a 5th force	$\mathcal{E}^2$
The seeds for galaxies	
(4) Primaevial adiabatic fluctuations	$\mathcal{E}$
(a) gaussian and scale-invariant	$\mathcal{E}^2$
(b) a specially chosen spectrum	$\mathcal{E}^4$
(5) Primaevial isocurvature fluctuations	$\mathcal{E}^4$
(6) Massive cosmic string loops	
(a) $G\mu$ to make something	$\mathcal{E}^2$
(b) $G\mu$ needed for accretion	$\mathcal{E}^3$
(7) Primaevial magnetic fields	$\mathcal{E}^2$
The development of galaxies	
(8) Gravitational instability	$\mathcal{E}$
(9) Explosive amplification	$\mathcal{E}^2$

tron annihilation in the early Universe then the present mean neutrino number density would be $n_\nu \approx 100 \text{ cm}^{-3}$, and if the neutrino mass were $m_\nu \approx 30 \text{ eV}$ the product $m_\nu n_\nu$ would make $\Omega = 1$. Other kinds of postulated dark matter particles are called cold dark matter (CDM, a designation introduced by R. Bond and A. Szalay) because their *primaeva* velocities are assumed to be much less than that of the 30-eV neutrinos, which have relativistic speeds when they decouple. We assign probability $\mathcal{E}^2$ that $\Omega = 1$ due to massive neutrinos (the hot dark matter, or HDM hypothesis), because we know neutrinos exist; we add another factor of $\mathcal{E}$ both for CDM, because the existence of the candidate particles is not demonstrated, and also for $\Omega = 1$ in baryons, because of the difficulty of hiding all those baryons⁵.

We must consider next the nature of the *primaeva* density fluctuations that existed in the very early Universe. The adiabatic mode of fluctuations, in which the local number density of photons is proportional to the number density of baryons and to the number density of dark matter particles, so that any fluctuation represents a local change in cosmological energy density, is generally considered the prime candidate for seeding large-scale structure. Adiabatic perturbations are generic in the sense that if in the very early Universe the number densities of photons, baryons and dark matter particles were perturbed from homogeneity by independent but comparable fractional amounts, the adiabatic combination would grow by gravity to become dominant at much later times. In cosmologies with inflation, adiabatic perturbations emerge naturally (but not inevitably^{6,7}) from quantum fluctuations of the fields that drive inflation³. In these models, baryons must be created out of entropy after the last serious bout of inflation. If the baryon creation efficiency is universal, independent of position, then the local number density of baryons is proportional to the local number density of photons⁸, which is the adiabatic condition. Therefore we assign probability $\mathcal{E}$ to the assumption that large-scale structure grew out of *primaeva* adiabatic fluctuations. This is as confident as we feel about any speculation concerning the physics of the early Universe.

The choice of adiabatic fluctuations does not take us very far; we need to specify how the density fluctuations vary with position. Inflationary physics³ provides us with some very reasonable-looking hints that may be more general than the specific models we have now. If the Universe is perturbed by quantum fluctuations of a field that drove inflation, then the fluctuations must have been very small to avoid over-perturbing the Universe, so it is reasonable to expect that they are well described by a free field. This would produce a gaussian (or random phase) process. The popular choice for the power spectrum of the fluctuations is the scale-invariant case, where the r.m.s. mass fluctuation on any scale is constant at the moment that scale crosses the cosmological horizon. In inflationary cosmologies, the scale-invariant spectrum is a consequence of the symmetry of the inflationary epoch to time-translational invariance, all scales being treated democratically³. The combination of gaussian mass fluctuations with a scale-invariant spectrum having the right amplitude to make galaxies and clusters of galaxies is aesthetically pleasing but places a very specific demand on highly uncertain physics. We therefore assign it a lower probability than for the assumption of adiabatic fluctuations alone, $\mathcal{E}^2$.

Table 2 A cosmic book: the models

Baryon adiabatic (2c, 4b, 8)	$\mathcal{E}^8$
Hot dark matter (2a, 4b, 8)	$\mathcal{E}^7$
Canonical cold dark matter (2b, 4a, 8)	$\mathcal{E}^6$
Cosmic string loops (2b, 6b, 8)	$\mathcal{E}^7$
with HDM (2a, 6b, 8)	$\mathcal{E}^6$
with no exotic matter (1, 6b, 8)	$\mathcal{E}^5$
Magnetized strings (2b, 6b, 7, 9)	$\mathcal{E}^{10}$
with no exotic matter (1, 6b, 7, 9)	$\mathcal{E}^8$
Baryonic isocurvature (1, 5, 8)	$\mathcal{E}^6$

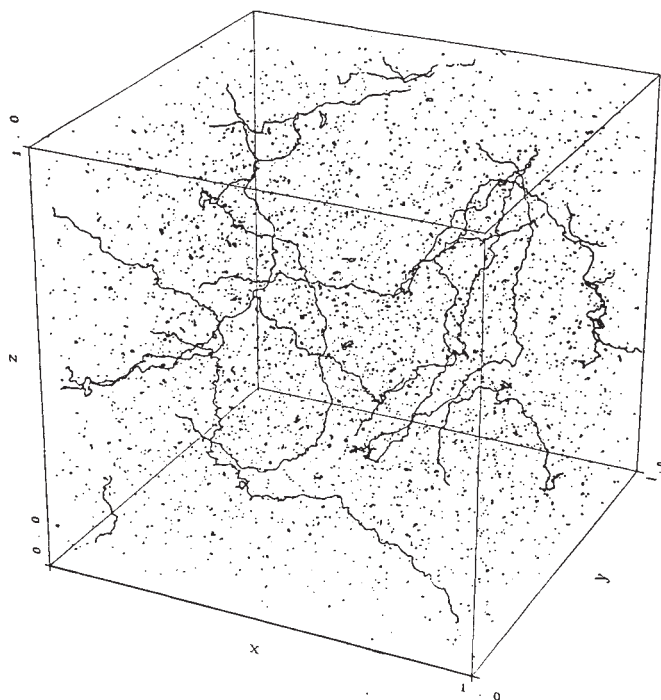


Fig. 1 A cosmic string simulation, from ref. 40. A cube containing a region of dimension equal to half the observable Universe is displayed.

Isocurvature fluctuations, where cosmological density is constant but there is a local variation in the ratio of photon to baryon number, or equivalently the entropy per particle, provide a mode orthogonal to adiabatic fluctuations. Scale invariance is not at issue here because at very early epochs, mass density fluctuations are supposed to be negligible. If the entropy per baryon number is the random variable, then to avoid excess microwave background anisotropy, one must assume that the power spectrum is nearly flat^{9,10}. There have been some discussions of how isocurvature perturbations could arise in inflation^{6,7}, but none come close to predicting the required spectrum. We therefore assign a low probability, $\mathcal{E}^4$, that nature somehow found a way to achieve this goal.

Cosmic strings provide an alternative source of density fluctuations. These topological defects from the symmetry-breaking phase transition at the end of the grand unification epoch ($T \approx 10^{16} \text{ GeV}$) are acausal line-like domains of relic vacuum that act like isocurvature perturbations of the Friedmann Universe. Strings are incompatible with standard inflationary models, but could arise in reasonable variants of inflation. For example, strings could appear as a consequence of a second symmetry-breaking phase transition at the end of the inflationary phase. The beauty of cosmic strings is that they are described by a single parameter, namely the energy scale of the Universe when they are created. This scale sets their mass per unit length μ to be about $10^{-6} G^{-1} c^2$, where G is the gravitational constant and c the speed of light. Strings are initially infinite, but as they are overtaken by the horizon and become causally connected, they whirl around at light speed, tangle, and break up into many smaller loops (Fig. 1). These loops eventually decay away by emission of gravitational radiation. Timing observations of millisecond pulsars can probe the gravity wave background over wavelengths of order of 1 light yr, and are presently on the verge of detecting or ruling out the cosmic string hypothesis^{11,12}. Loops of cosmic string act as non-linear seeds that gravitationally accrete mass during the matter-dominated era¹³⁻¹⁵. We assign a probability $\mathcal{E}^2$ for the existence of cosmic strings that would do something of interest, and an extra power of $\mathcal{E}$ for the probability

that the mass per unit length, μ , is the value required if string loops are to form galaxies by gravitational accretion. This brings the probability to the same level as for the CDM hypothesis, which seems reasonable.

Broad classes of cosmic strings are superconducting. If a primordial magnetic field existed, with energy density roughly comparable to that of the cosmic background radiation, then superconducting strings would generate huge currents as they thrash around during their evolution as oscillating loops. This would be a potent generator of energy for explosively driven blast waves that can pile up matter into shells that could fragment to form galaxies in sheet-like clusters¹⁶. No known mechanism can account for the wanted primordial field, but one cannot afford to be too dogmatic about its existence. We assign a probability of $\mathcal{E}^2$ to the existence of a primordial magnetic field with interesting field strength. That this is about the same probability as for the HDM hypothesis seems reasonable, because cosmic magnetic fields, like neutrinos, are observed.

Galaxy formation can proceed either via gravitational instability developing out of primordial mass-density fluctuations in the early Universe or by hydrodynamic processes. An example of the latter is explosive amplification: matter accumulated by blast waves into thin dense shells is unstable to fragmentation, and the fragments may in turn develop blast waves producing a new generation of more massive fragments^{17,18}. We assign a probability $\mathcal{E}$ to the gravitational instability process, because we seem to have an example of the process in operation in the growth of the Local Supercluster of galaxies¹⁹. We assign probability $\mathcal{E}^2$ to the explosive amplification scheme because examples of this process in the interstellar medium operate under such a different scale of conditions from that of galaxy formation.

The models

Table 2 lists our joint probabilities for six models for galaxy formation. The numbers in parentheses are the main elements from Table 1. The first model, the gravitational development of adiabatic perturbations in a Universe now dominated by baryons, is very tightly constrained by its tendency to overperturb the microwave background and by its tendency to make the first generation of objects too large²⁰. It may be possible nevertheless to adjust the primaeval spectrum of density fluctuations to avoid a direct conflict with the observations. If so, we arrive at probability $\mathcal{E}^8$ for this model.

The massive neutrino model was the first to take account of the possibly key role of non-baryonic matter. The standard version is described by Zel'dovich *et al.*²¹. Perhaps the greatest success of the model is the prediction that galaxies tend to lie on sheets, as observed in the redshift surveys^{22,23}. If one assumes a scale-invariant spectrum (4a in Table 1) and also that galaxies trace mass on large scales, then the amplitude of the spectrum cannot be adjusted to make galaxies form at a reasonable epoch without overproducing large-scale fluctuations in the galaxy distribution^{24,25}. We consider this a serious but certainly not fatal problem. One way out is to assume that mass clusters more strongly than galaxies, which raises the amplitude of the power spectrum²⁶. We are not very optimistic about the chances for this approach because to reconcile the dynamical estimates of the mass density with the Einstein-deSitter model one needs biasing in the opposite direction, with galaxies more strongly clustered than mass¹. Another possibility is to go to isocurvature perturbations^{27,28}. Cosmic strings are a specific example of isocurvature-like perturbations that are reasonably well motivated by particle physics considerations. In the table, we have assumed that the power spectrum of adiabatic fluctuations can be adjusted to increase small-scale fluctuations to make galaxies form earlier. This brings the probability to $\mathcal{E}^7$.

The canonical cold dark matter model has been studied in great detail²⁹. This assumes adiabatic gaussian primaeval mass density fluctuations with a scale-invariant spectrum in an Einstein-deSitter Universe that is dominated by exotic cold dark

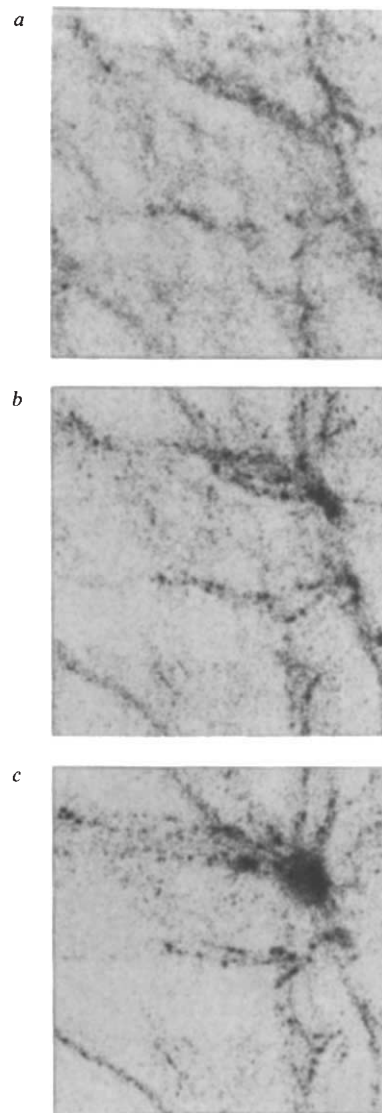


Fig. 2 A cold dark matter simulation, from ref. 56. A slice of the Universe in a box (a) 200, (b) 80 and (c) 40 Mpc on the side at the present epoch is shown in 2-dimensional projection. Initial conditions were appropriate to cold dark matter with density parameter $\Omega = 1$, Hubble constant $H = 100 h \text{ km s}^{-1} \text{ Mpc}^{-1}$ ($h = 0.5$), and 262,144 particles being placed in the initial cube. The correlation length is 10 Mpc and structure clearly extends over larger distances.

matter. Our joint probability for this model is $\mathcal{E}^9$. Where does it reveal any observational shortcomings? These are two suspect areas, on small (~ 0.1 Mpc) and very large (~ 50 Mpc) scales. The problem on small scales is overmerging: objects with the scale of galaxies do not seem to last very long, tending to fall together and merge into supermassive agglomerations. The result²⁹ is that the objects one might want to call galaxies have excessively extended halos at the present epoch, and, in particular, at redshifts $z \approx 1$ they look much more fragmented than galaxies actually observed at that redshift³⁰⁻³². Perhaps when dissipation is incorporated into the modelling, it will save the baryonic cores from merging and produce groups of galaxies which more closely resemble the observed structures.

On large scales, the simulations find enormous filaments of galaxies, extending over scales larger than the coherence length of the primaeval non-linear fluctuations. Is this a real effect, or could it be an artifact of the numerical models (Fig. 2)? There are great filaments of galaxies as long as 100 Mpc, as in Perseus-

Pisces²³, and their explanation would be a great success for any theory. Perhaps the most pressing challenge to cold dark matter arises from the observations of large-scale streaming motions at speeds of $\sim 600 \text{ km s}^{-1}$ (ref. 33). The reality of the large scale flows is critically dependent on the reliability of the distance indicator method. For example, noise in the zero-point calibration, which could arise from large-scale variations of modest amplitude on the galaxy mass-luminosity ratio, would result in an apparent cosmic drift. However, it is not clear to what extent such an effect would weaken the evidence for the dipole and quadrupole components of the large-scale flow. A vivid impression of the cosmic drift effect that to some extent circumvents this problem has been obtained from a redshift survey of a volume-limited sample of IRAS (Infrared Astronomy Satellite) galaxies³⁴. By assuming that mass traces light, the density field can be evaluated, and the peculiar gravity field predicted. The resulting vector field is shown in Fig. 3, projected onto the supergalactic plane. The relative length and directions of the vectors correspond to the inferred accelerations; their overall scale, however, is arbitrary. Great mass concentrations are seen that can produce a flow field that is coherent over tens of Mpc. A recent analysis by Regös and Szalay³⁵ of sampling bias in the galaxy selection function confirms the large-scale coherence of the peculiar velocity pattern (Fig. 4). These authors show that the large-scale density fluctuations present in the cold dark matter theory yield a comparable coherence pattern to that observed but the standard normalization, with biasing factor (ratio of luminous galaxy to dark matter correlations) of a few, leads to too small an amplitude. Bertschinger and Juszkwicz³⁶ point out that the probability in the cold dark matter model of finding even one 'great attractor', the mass concentration of $\sim 5 \times 10^{16} M_{\odot}$ required to account for the large-scale coherence in the velocity flow, is less than one per Hubble volume. Yet another critical issue is the cosmic microwave anisotropy. If the reported detection³⁷ of fluctuations in the microwave sky brightness at an amplitude $\delta T/T \approx 5 \times 10^{-5}$ on an angular scale of 8° is reproduced by measurements at a second frequency, one would have to admit a shortcoming in large-scale power in the CDM model by about an order of magnitude.

If in the cosmic string picture one invokes CDM¹⁵, our probability is $\mathcal{E}^7$. Currently, attention is turning to strings with HDM^{38,39}; this raises the probability to $\mathcal{E}^6$. If one could escape with $\Omega = 0.1$ in baryons, an option that may be viable, our probability would rise to $\mathcal{E}^5$, which is the best bet by our measure. We cannot yet assess the observational situation for any variant of the string picture because the theory has not been worked out in sufficient detail to confront the observations adequately. For example, Turok¹⁴ argued that the correlations among positions of clusters of galaxies were explained by cosmic strings, while beautiful new simulations⁴⁰ (Fig. 1) indicate that loop peculiar velocities may tend to erase the correlations. Another problem is that the galaxy seeds provided by the cosmic strings have a power-law distribution of seed masses¹⁴. How does this fit with the remarkable observation that there is a hard cutoff at about $10^{11} M_{\odot}$ in the mass found within radius $r = 5 \text{ kpc} \approx 1500 \text{ light yr}$ in a galaxy? A further complication is that the recent numerical simulations³⁹ suggest that cosmic loop formation is much more effective than hitherto realized, so that there are many (10–100) small string loops per galaxy. The implications for accretion and subsequent galaxy formation have yet to be studied. On the positive side, the non-gaussian nature of strings allows considerable freedom in reconciling the assumption of individual nearby loops that might be responsible for large peculiar velocities with the statistically small perturbations that loops are allowed to induce on the cosmic microwave background^{15,41} or the gravity wave background, as sampled by the millisecond pulsar^{11,12}. Cosmic string wakes may provide a natural means of seeding very large-scale structure^{15,42,43}. The ultimate test of cosmic string theory is likely to come with the search for its trace in the cosmic microwave background radi-

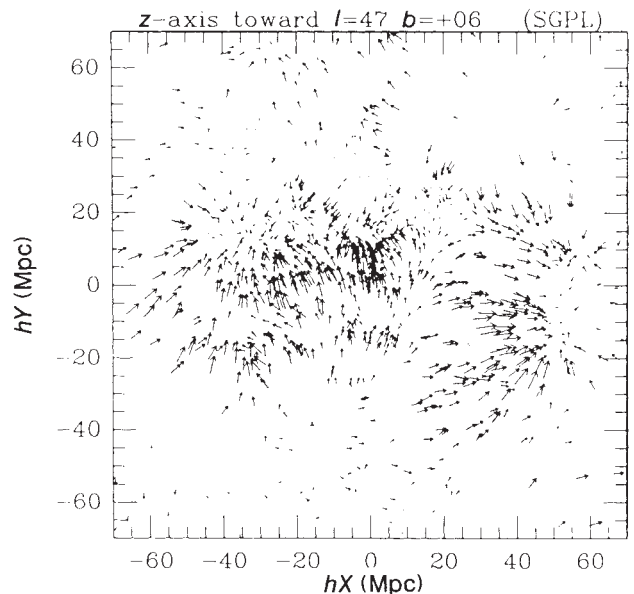


Fig. 3 The peculiar gravity field, from ref. 34. The vectors indicate relative strength and direction of the gravitational acceleration in the supergalactic plane, as predicted from an IRAS volume-limited galaxy sample. Our galaxy is centrally located, Virgo is the concentration just above us, Perseus-Pisces is on the right, the great attractor is in the upper left and Coma is at the top.

ation⁴⁴. The temperature fluctuations on degree scales are predicted to be of order 10^{-5} , and have a characteristic non-gaussian signature.

The original version¹⁶ of the magnetized cosmic string picture has a probability of $\mathcal{E}^{10}$, the record high power among the models surveyed. Ostriker *et al.* recognized that the picture may well work also with baryons alone; that would yield $\mathcal{E}^8$. On the observational side, the model has some impressive virtues. The required primaeval magnetic field is about what would be expected from scaling the field strength in the interstellar medium if flux were conserved (under isotropic compression), although this only begs the question of the origin of such a field. It may require either primordial density fluctuations⁴⁵ or even the protogalactic environment⁴⁶ to generate such a field via a dynamo, although specific superconducting string models may be capable of driving a self-induced dynamo⁴⁷. The model leads to reasonable galaxy baryon masses and accounts for the observed correlations among positions of clusters of galaxies by a picture involving overlapping spheres produced by the blast waves, with clusters forming at triple intersection points⁴⁸. The model also predicts distortions of the cosmic microwave background spectrum at the few percent level, as a result of energy injected in the early Universe by the oscillating magnetized strings⁴⁹. A somewhat larger effect (of order 10%) is actually reported in the recent Berkeley-Nagoya spectrum measurements⁵⁰. Apart from the issue of plausibility, an observational point on which this theory may flounder is the uniform motion of our Local Group of galaxies and the surroundings out to distances $\sim 10 \text{ Mpc} \approx 30 \text{ million light yr}$ at a speed of some 600 km s^{-1} relative to the cosmic background radiation, which seems unlikely to be blast-wave induced⁵¹. In addition, there is a potential problem with galaxy formation. Explosions result in fast-moving baryonic cores, candidates for ellipticals and the spheroid components of spirals. But can massive dark halos with cores of size $\sim 10 \text{ kpc}$ form by secondary infall of exotic dark matter around such rapidly moving cores? The co-moving scale over which such accretion occurs is at least 100 kpc , and it would seem difficult to form a more compact halo without invoking dissipation of the dark matter.

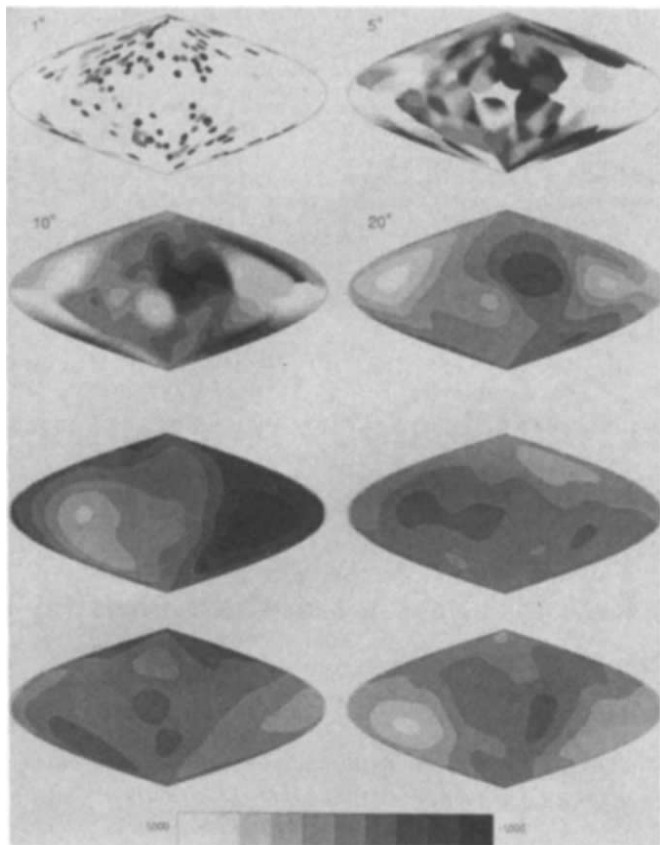


Fig. 4 (From ref. 35.) Top, smoothed velocity field of the elliptical galaxy data of ref. 33, with distances out to $8,000 \text{ km s}^{-1}$, corresponding to an effective gaussian radius of about $3,000 \text{ km s}^{-1}$. Each galaxy has been smoothed with a gaussian filter with radius 1, 5, 10, 20° respectively, cut off at 4 filter radii, and the regions with no galaxies within that distance were left blank. Velocities are coded with the gray scales spanning the range of $\sim 1,000 \text{ km s}^{-1}$ through $1,000 \text{ km s}^{-1}$, and the small bins are 200 km s^{-1} wide. Light shades correspond to redshift, dark to blueshift. Only the first 10 harmonics are summed, and one should compare the 20° filtering with the Monte-Carlo pictures below. Bottom, realizations of the velocity field in the unbiased CDM scenario, where the survey size is limited to $3,000 \text{ km s}^{-1}$, and the selection function is error weighted; normalization is the same as above. A total of 16 different random cases have been created. Of these 16 models, about two have a large dipole of about 600 km s^{-1} in magnitude, as do the data, but the others have much smaller velocities. Velocities can be scaled to any biasing, but with a biasing factor of a few such a large dipole would be extremely unlikely. Here we show four representative simulations.

The only currently popular theory that commences with the observed mass density ($\Omega \approx 0.1$) requires isocurvature perturbations with a suitably chosen spectrum. Our joint probability here is $\mathcal{E}^6$. The baryon isocurvature model does not appeal to exotic forms of dark matter, and its primordial spectrum is dominated by the maximum baryon Jeans mass of $\sim 10^{17} M_\odot$. It leads rather naturally to structures such as the great attractor, produces large cosmic streaming, and makes galaxies very early, at redshifts $z \approx 30$ (refs 9, 52). The only rival model which appears to meet these requirements is that of cosmic strings: string loops are non-linear seeds that accrete throughout the matter-dominated era, can be observable as early as $z \approx 1,000$, and can even lead to objects reminiscent of the great attractor⁵³. Perhaps a combination of $\Omega \approx 0.1$ in baryons and cosmic strings yields a model with desirable attributes, the strings providing a more or less natural source of the needed isocurvature perturbations.

This leads us to the fascinating question of the epoch of formation of galaxies. Some galaxies appear to have formed

recently, at $z \lesssim 1$, as judged by the enormous star formation rates implied by the infrared luminosities⁵⁴. On the other hand, other bright galaxies observed at redshifts $z \approx 1-3$ have infrared luminosities suggestive of relatively old star populations³⁰⁻³². Also suggestive of early formation of stars, if not galaxies, is the observation that the intergalactic medium as probed by ultraviolet radiation from quasars seems to have been in place at $z \approx 4$ (ref. 55). Our impression is that if assembly of the bright parts of galaxies were not complete by a redshift well above $z \approx 3$, it would be a severe problem for the baryon isocurvature model and for the string models, and that if galaxies were assembled at $z \approx 2$ or earlier, it would present a severe problem for the CDM model. It does not seem too optimistic to hope that the observations will be capable of deciding between these possibilities in the next few years.

Conclusions

The key to our cosmic book is the base probability, $\mathcal{E}$. In our most optimistic mood, we take $\mathcal{E} \approx 0.35$ because that makes the probabilities in the first section of Table 1, for the cosmological model, add to unity. This means we are assigning a probability of only 10% to the chance that galaxy formation depends on some new element of physics. That is consistent with standard and sensible methodology: for the most part, one should do research under the assumption that all the essential pieces of the puzzle are on hand, while bearing in mind that history shows that that is not always so.

It is reasonable that the sum of the probabilities in Table 2 should be well below unity because we are attempting to unravel a very complex puzzle with the help of a meagre fund of clues from observations. With $\mathcal{E} = 0.35$, the sum of probabilities for the models is 0.01. These non-negligible odds should make cosmologists happy while cautioning that we are engaged in a highly speculative pursuit. We may feel tempted to tack on such probability-raising measures as the anthropic principle, or multiplication of the number of candidate models by a scatter-shot approach. This should be our last resort, however. For now, we can enjoy the spectacle as theoretical and observational discoveries continue to press and stimulate cosmological theories ever harder.

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ARTICLES

The HIV *tat* gene induces dermal lesions resembling Kaposi's sarcoma in transgenic mice

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When the human immunodeficiency virus transactivating gene under the control of the viral regulatory region is introduced into the germline of mice, skin lesions are induced that resemble Kaposi's sarcoma seen in AIDS. Our findings indicate that HIV could play a direct part in causing cancer.

THE human immunodeficiency virus (HIV) has been implicated in various clinical manifestations associated with AIDS, including the acquisition of an immunodeficient state, the frequent presence of neurological disorders, and an array of malignancies^{1,2}.

It is tempting to explain the multiple manifestations associated with HIV infection by considering the effects of the virus on the different cell types in which it is found. This virus has a demonstrated predilection for cells that express the CD4 surface antigen^{3,4}. A number of cell types express variable levels of the CD4 receptor, as shown by immunocytochemistry with anti-CD4 antibodies or hybridization with CD4 DNA probes. There is also direct evidence for the presence of HIV in most of these cells, including both T and B cells, cells in the macrophage-monocyte lineage, neural cells such as neurons, oligodendrocytes, astrocytes and microglial cells, and possibly endothelial cells as well⁵⁻⁸. Many of these cell types exist in a variety of tissues and HIV-induced dysfunction of these cells could affect the tissues where they are found.

Malignancies associated with AIDS include Kaposi's sarcoma, B-cell lymphoma, squamous cell carcinoma and carcinoma of the rectum⁹. Kaposi's sarcoma is an initial manifestation of AIDS in ~25% of cases, and autopsy series have reported cutaneous as well as visceral and lymph node involvement in ~50% of individuals^{9,10}. Its increased incidence in male AIDS patients who are homosexual may implicate other sexually transmitted agents such as cytomegalovirus, or certain practices such as the use of inhaled nitrites, in its aetiology¹⁰. AIDS-associated lymphomas, which are commonly found in the central nervous system, exist in 10-20% of autopsy cases⁹. There is currently no evidence for HIV directly inducing any of these malignancies, and most could result from either the underlying

immunodeficient state of the infected individuals or to environmental factors associated with specific risk groups.

HIV is a retrovirus in the lentivirus subfamily¹. Apart from *gag*, *pol* and *env*, it contains at least six other genes, designated *tat*, *rev* (*art/trs*), *vif* (*sor/orfA*), *nef* (*3'orf/orfB*), *vpr* (*R*) and *vpu*, which are important for regulating viral gene expression and replication. The product of the *tat* gene is a potent transactivator, capable of up-regulating viral gene expression in cultured cells by both transcriptional and post-transcriptional mechanisms¹¹⁻¹⁶. The target sequence for *tat* transactivation has been localized within the long terminal repeat (LTR), immediately downstream of the messenger RNA start-site¹⁷.

As the product of the *tat* gene acts to control viral gene expression, it may also regulate cellular gene expression which will lead to specific disease(s) associated with HIV infection^{18,19}. To test this possibility, we have introduced the *tat* gene under the control of the HIV LTR into the germline of mice. The availability of such transgenic animals will allow us to determine not only the tissue specificity of the viral LTR, but also the biological effects of the *tat* gene in a whole intact animal where there is a complex interplay between different cell types.

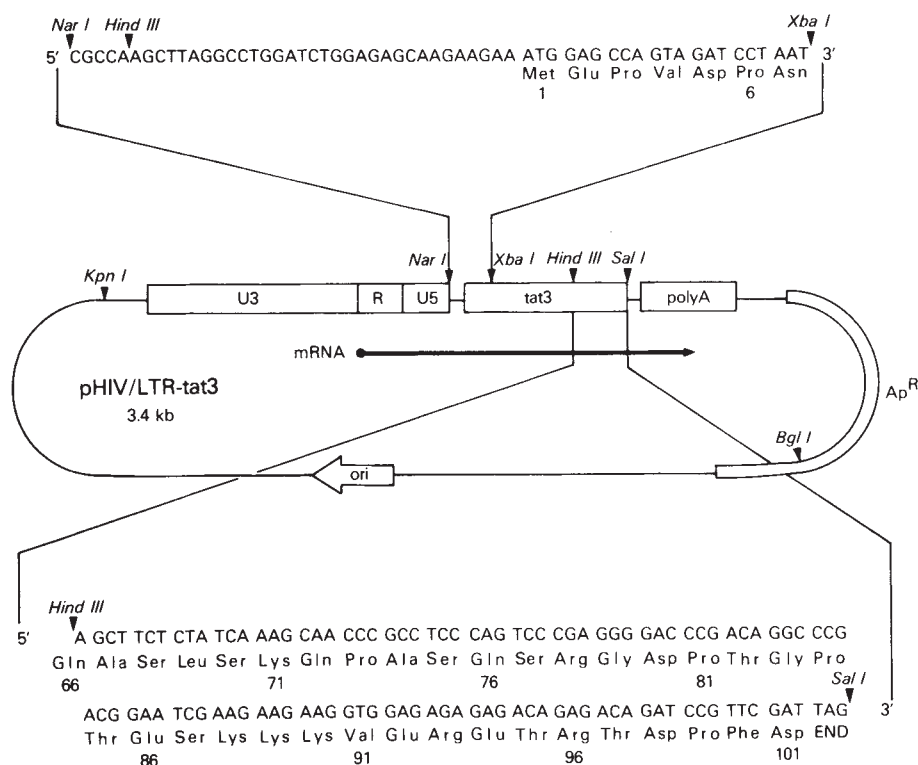
Construction of pHIV/LTR-*tat3* plasmid

The pHIV/LTR-*tat3* expression vector was constructed by placing the proviral 3' LTR and the authentic *tat* sequence from the AIDS-associated retrovirus strain 2 into the pML cloning vector (Fig. 1). The LTR sequence is complete and contains the negative regulatory element and the positive regulatory element, with the transcriptional enhancer¹⁷. The LTR also includes the TAR sequence which is located within the R region and functions as the target for *tat* transactivation. The *tat* sequence was derived by taking the 179-base pair (bp) *XbaI-HindIII* fragment, encoding amino acids 8-66, from ARV-2 and completing the missing 5' and 3' coding sequences with synthetic oligonucleotides (Fig. 1). The entire unspliced sequence was placed immediately downstream of the LTR sequence and upstream

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Fig. 1 Features of the pHIV/LTR-tat3 plasmid. The *tat* coding sequence was derived by taking a 179-bp *Xba*I-*Hind*III fragment from the cloned HIV ARV-2 genome and completing the missing 5' (top) and 3' (bottom) sequences with the indicated synthetic oligonucleotides. This entire coding sequence has been placed downstream of the HIV ARV-2 LTR, including the U3, R, and U5 regions, and upstream of a fragment containing the SV40 polyadenylation site. This chimaeric gene has been inserted into the pML vector and the resulting plasmid is 3.4 kb in size. The mRNA expected from this transcriptional unit is indicated.



of the SV40 polyadenylation site. In this chimaeric construct, transcription is expected to initiate at the beginning of the R region, proceed across the *tat* coding sequence, and terminate within the SV40 poly(A) site. The *tat* gene product from ARV-2 contains 101 amino acids, as opposed to *tat* from HTLV-III_B or LAV, which contains 86 residues²⁰⁻²². The additional amino acids from ARV-2 are located in the carboxyl terminus, which has been shown by transfection studies to be dispensable for transactivation of the viral LTR²³.

Derivation of transgenic mice

To generate transgenic mouse lines carrying the LTR-tat3 sequence, a 2-kilobase (kb) *Kpn*I-*Bgl*I fragment containing the complete transcriptional unit was purified from pHIV/LTR-tat3 and microinjected into fertilized eggs from superovulated CD-1 females (Charles River Laboratories), which are outbred mice previously mated to (C57BL/6 × DBA/2) F₁ males (Jackson Laboratories)¹⁸. After screening offspring for intact LTR-tat3 sequences by Southern blot hybridization, six founder mice were identified. In order to select three founder animals for establishing transgenic lines, the tails of the six founder mice were checked for the expression of the ~0.8-kb *tat* mRNA. The three founders, designated C4, E10 and F2, with the highest levels of expression were maintained by crossing with outbred CD-1 mice. As similar results were obtained in all three founder lines studied, the observations we describe here must be due to the expression of the *tat* gene, rather than to the specific site of integration of the transgene.

Expression of the *tat* gene in the skin

To determine where the LTR-tat3 sequence was expressed in these transgenic mice, tissues were taken from representative F₁ offspring of each founder line and analysed for *tat* mRNA expression by Northern blot analysis. Of the tissues studied, only skin gave the expected 0.8-kb mRNA in all three founder lines (Fig. 3). The remaining tissues included brain, thymus, heart, lung, liver, intestine, kidney, pancreas, spleen, muscle and testes, and did not express detectable levels of the *tat* mRNA.

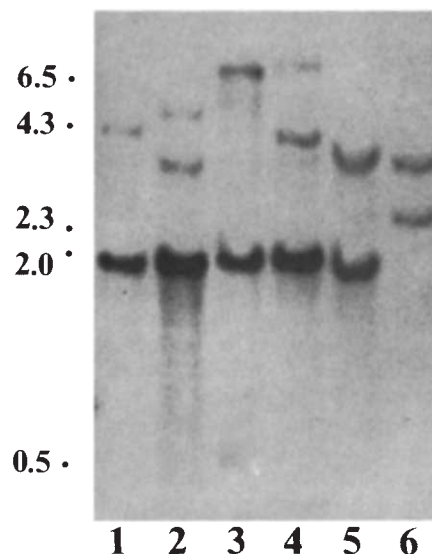


Fig. 2 Identification of transgenic *tat* mice. Tail DNA from each of the six founder mice was compared by Southern blot analysis. Lane 1, mouse B6; lane 2, mouse C4; lane 3, mouse E10; lane 4, mouse F2; lane 5, mouse H2; and lane 6, mouse F5. Except for mouse F5 (lane 6), which contained one or less than one copy of the transgene, all other founder mice showed a band of ~2 kb in unit length and presumably derived from a single integration site containing multiple copies of the *tat* gene in a tandem array. The extent of hybridization seen with DNA from mouse B6 (lane 1) represents about 2 copies of the transgene per cell. The other bands probably represent host flanking sequences that are connected to the transgene. The flanking sequences differ between transgenic mice. The molecular size markers used are shown on the left in kilobases.

Methods. DNA (15 µg) was digested with *Bgl*II, which cleaves only once within the chimaeric gene, then fractionated on a 1% agarose gel and transferred to a nitrocellulose membrane⁵¹. Hybridization was performed with an M13 DNA probe containing the *tat* sequence.

The expression observed in the tail was due entirely to the overlying skin.

Both males and females expressed roughly equivalent levels of *tat* mRNA in the skin, although individual variation in the level of expression was noted between different animals in the same founder series (data not shown). Neither the age of the animal, nor the number of copies of the transgene in the genome correlated with the level of *tat* expression.

The reason for the absence of expression in tissues apart from the skin is not obvious. An analogy could possibly be drawn to the latent state of HIV infection²⁴⁻²⁷, where the proviral sequence is presumably present in a variety of cell types but viral genes are not expressed unless activated by a variety of physical, chemical and biological agents²⁸⁻³⁴.

Dermal proliferation of cells

A careful histological examination of the back skin was then undertaken in F₁ mice of about 4 months old. The most prominent finding was hypercellularity of spindle-shaped cells in the dermis of male mice. Figure 4a shows normal back skin from a negative male littermate. The epidermis shows normal maturation and is uniform in thickness. The earliest lesions of the skin from a transgenic male littermate show epidermal hyperplasia, with accentuation of the granular layer and slight hyperkeratosis (Fig. 4b). Increased cellularity of the dermis is present with proliferation of fibroblastic cells along the dermal-epidermal junction. The overlying epithelium is intact and without ulceration.

Increasing cellularity in the papillary dermis, composed primarily of spindle-shaped cells with elongate cytoplasmic processes and oval nuclei, is seen in another transgenic mouse (Fig. 4c). Inflammatory cells are present throughout the dermis, as well as extending through the epidermis. Pigment-laden macrophages and extravasated red blood cells (RBCs) are scattered throughout the dermis. An advanced lesion in a 12-month-old male transgenic mouse is shown in Fig. 4d. There is marked hypercellularity of the dermis and replacement of collagen by spindle-shaped or fibroblastic cells. Also present are scattered vascular spaces containing RBCs. Whereas the nuclei of early lesions are dense, the nuclear chromatin of later lesions is more open with prominent nucleoli. The histological appearance of these changes in the skin was suggestive of the early patch-type lesions of Kaposi's sarcoma seen in patients with HIV infection.

Trauma from fighting was not considered to be a factor in the aetiology of these lesions because isolated animals also had identical skin changes. In addition, we saw these lesions in animals which had had no previous skin biopsies. Interestingly, although these lesions are present to varying degrees in most male transgenic animals (33/37) from each of the three founder lines, they were not seen in any of the female transgenic mice (0/15) or in any of the negative control male littermates (0/10).

Multicentric tumours in the dermis

At 12 to 18 months of age, ~15% of the male mice developed skin tumours. Most of these animals had not had a skin biopsy. Of the 10 mice that developed tumours, 4 had tumours at more than one site. Figure 5a shows an animal with extensive and diffuse tumours over both the left and the right shoulders, as well as a circumscribed and defined tumour nodule on his right flank; some of these tumours are markedly erythematous. Histologically their appearance suggests a progression or a derivation from the focal proliferation of spindle cells seen in earlier lesions. Again, such lesions were never seen in female littermates carrying the *tat* gene.

Another characteristic finding in at least 6 of the male transgenic mice was a propensity for spontaneous haemorrhage into the skin without any obvious precipitating aetiology. These haemorrhagic lesions may easily be missed because they have a tendency to resolve over time. The areas of haemorrhage were not usually associated with tumour nodules. The extent of the

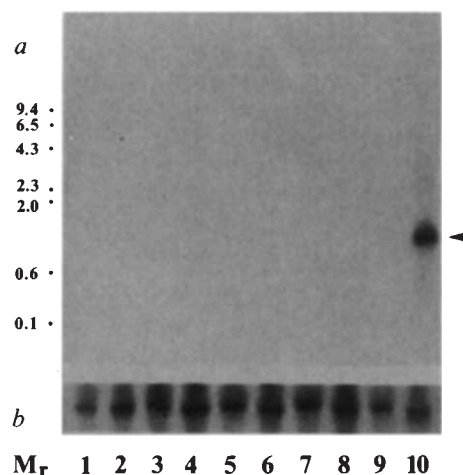


Fig. 3 Tissue-specific expression of the LTR-*tat3* gene. Poly(A)⁺ RNA from different tissues was compared by Northern blot analysis. *a*, Hybridization with an M13 DNA probe containing the *tat* sequence. *b*, Hybridization of the same blot with an M13 DNA probe directed against the major histocompatibility complex class I genes to indicate that equivalent amounts of RNA from each tissue were analysed. Lane 1, brain; lane 2, thymus; lane 3, heart; lane 4, lung; lane 5, liver; lane 6, intestine; lane 7, kidney; lane 8, pancreas; lane 9, spleen; and lane 10, skin. The molecular size markers shown on the left in kilobases were derived from double-stranded DNA.

Methods. Total RNA was obtained from different tissues of an E10 F₁ transgenic mouse by the isothiocyanate-caesium chloride procedure. Poly(A)⁺ RNA was fractionated on a 1% agarose-formaldehyde gel and transferred to a nitrocellulose membrane⁵².

haemorrhage could either be localized (Fig. 5b) or could involve large diffuse areas of skin (Fig. 5c). This was not seen in either female transgenic littermates or age-matched negative control male littermates.

Histological analysis of these dermal tumour nodules was performed to see if they were similar to skin lesions such as Kaposi's sarcoma. The tumours arose from the dermal layer and contained spindle-shaped cells. In more aggressive undifferentiated lesions, there was considerable nuclear pleomorphism and frequent mitotic figures. Figure 5d shows a high-power view of an early lesion from a 10-month-old male mouse. In this case, slit-like spaces containing RBCs are already seen in the dermis, immediately underlying the hyperplastic epidermis. Figure 5e shows a portion of a tumour from a 12-month-old male. Multiple slit-like spaces scattered between the spindle-shaped tumour cells are seen. Extravasated RBCs are prominent. The capillary spaces are irregular and disorganized and do not appear to be continuously lined by well defined endothelial cells.

One aggressive tumour from a 14-month male mouse is shown in Fig. 5f. It consists of multiple mitotic figures and dense pleomorphic nuclei. In addition, a neoplastic vascular structure is present and consists of abnormally large, pleomorphic endothelial cells which line the capillary space. Some of the tumours may also have regions where the neoplastic cells have a fusiform appearance with curved or S-shaped nuclei. On occasion, they may include nerve roots and inflammatory infiltrates (Fig. 5g). Such characteristics may suggest the diagnosis of dermatofibroma or neurofibroma.

Lack of *tat* mRNA in tumour cells

We have shown that the *tat* gene is expressed in the skin and by Northern blot analysis have confirmed that the skin tumours expressed the *tat* gene, but it is possible that the *tat* mRNA was not expressed specifically in the tumour cells, but rather in other cells of the surrounding skin. As it was relatively easy to derive

Fig. 4 Progressive dermal lesions in the transgenic mice. Microscopic examination of the back skin from F_1 mice of different transgenic lines. Paraffin-embedded sections of fresh biopsies were stained by haematoxylin and eosin. *a*, Negative mouse at 4 months. *b*, E10 F_1 mouse at 4 months. *c*, C4 F_1 mouse at 4 months. *d*, E10 F_1 mouse at 12 months.

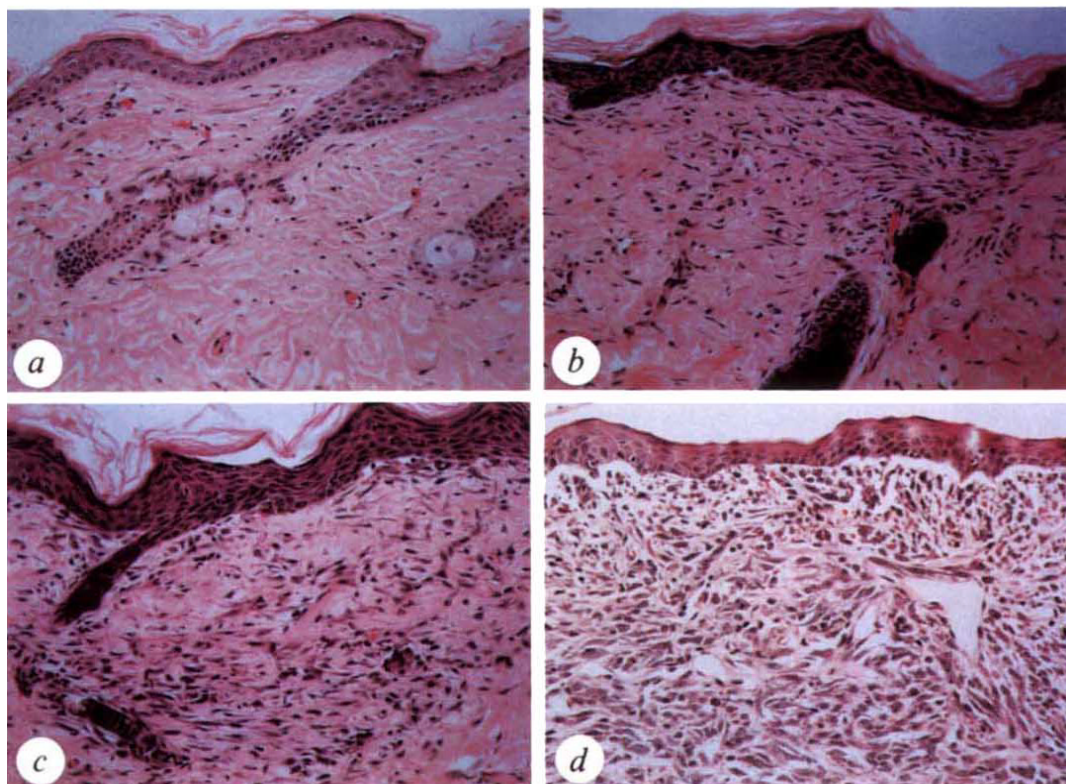
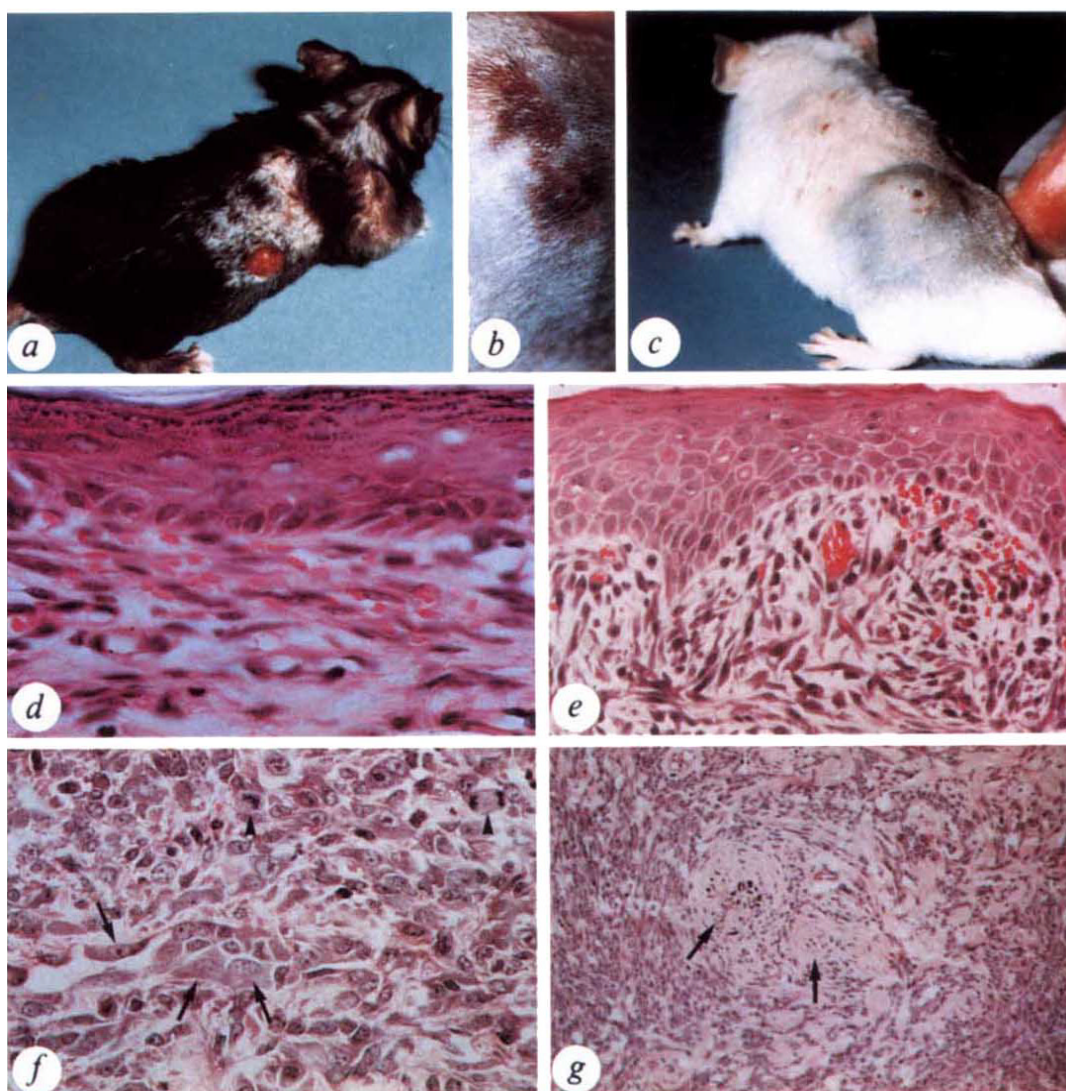


Fig. 5 Characterization of skin lesions and tumours in the transgenic mice. Gross and microscopic examination of lesions from F_1 mice. *a*, 14-month-old E10 F_1 mouse showing erythematous tumours on the right shoulder and the right flank. *b*, Close-up of an 8-month-old C4 F_1 mouse with a localized haemorrhage in the back. *c*, 10-month-old F2 F_1 mouse with diffused haemorrhage in the skin in the lower back area. *d*, Haematoxylin (H) and eosin (E) section of an early lesion from a 10-month-old E10 F_1 mouse showing slit-like spaces with RBCs in the dermis. *e*, H and E section of a tumour from a 12-month-old E10 F_1 mouse showing multiple slit-like spaces scattered with extravasated RBCs and spindle-shaped tumour cells. *f*, H and E section of a tumour from a 14-month-old E10 F_1 mouse, showing abnormally large pleomorphic endothelial cells (arrow) and multiple mitotic figures (arrowheads). *g*, H and E section of a tumour from a C4 F_1 mouse showing neoplastic cells around nerve roots (arrows).



cell lines from the aggressively growing tumours, we have examined whether *tat* gene expression could be detected in relatively early passage cells derived from three independent tumours. Using Northern blot analysis, we have not been able to demonstrate *tat* expression in any of the cell lines analysed (data not shown), although it may be below our limit of detection. Indeed, a high level of expression of *tat* may not be required once transformation has been established.

Mechanisms of tumour development

Multifocal areas of dermal hypercellularity and the subsequent development of malignant tumours were correlated with the expression of *tat* gene product in the skin of male mice. This phenotype cannot be due to the HIV LTR alone because the same phenotype occurs in different founder mouse lines which apparently have different integration sites, and because the HIV LTR alone does not give a phenotype³⁵.

The mechanism of tumorigenesis induced in the skin by the *tat* protein could be clarified by determining which cell types in the skin express the *tat* gene product. The *tat* gene product could exert its effects on the neoplastic cells either directly if expressed in these neoplastic cells, or indirectly if expressed in non-neoplastic cells in the skin by inducing mediators such as growth factors. As the cell lines derived from the dermal tumours do not express detectable levels of *tat* mRNA by Northern blot analysis, an indirect effect seems likely. It is possible then that accessory cells in the skin, such as Langerhan's macrophages, serve as cellular targets for the expression of the *tat* gene³⁶. These cells may in turn induce other cell types in the skin to proliferate, giving rise to the focal lesions seen in all male mice. These proliferative events may slowly progress, but will not develop into malignant tumours until a second genetic event intervenes. It is unlikely that these tumours resulted from a dysfunction of the immune system because we have not detected *tat* gene expression in any of the lymphoid tissues, nor have we detected changes in either the absolute number of T cells or the ratios of the T cell subsets. Regardless of the mechanism, the ability of the HIV *tat* gene to induce tumours in mice would suggest that HIV is a cancer-causing virus.

Resemblance to Kaposi's sarcoma

In our murine transgenic model, the dermal lesions associated with *tat* expression in the skin have a number of significant similarities to Kaposi's sarcoma (KS)³⁷⁻⁴³, which is of special interest because of its high frequency in autopsy series of AIDS patients. (1) A characteristic of the histopathology of KS is its progressive nature, beginning with early patch- and plaque-like lesions, and progressing to nodular lesions^{37,38}. Skin biopsies of transgenic male mice performed at different ages showed similar progressive characteristics. (2) KS is a unique multifocal neoplasm which is probably of polyclonal derivation⁴⁰. Virtually all our transgenic mice also showed multifocal hyperplastic lesions in the dermis and the subset that eventually developed neoplasms often had multicentric tumours of variable shapes in the skin. (3) KS lesions usually present as discrete, red, erythematous nodules^{37,39}. Some of the lesions in our mice also consisted of erythematous nodules, presumably as a result of their vascular nature. Local haemorrhage has been seen as a complicating feature in patients with KS lesions⁴⁴. We also noted a propensity for spontaneous haemorrhage in our male transgenic mice. The aetiology of this is not clear, but may result in part from underlying abnormalities of vascular structures. (4) There is a significant male preponderance in KS associated with HIV infection^{37,40}. However this could be explained by the prevalence of HIV infection among homosexual or bisexual men in the United States. But other varieties of KS, such as the classical form seen in elderly whites of Mediterranean descent and the endemic African form, also have a male preponderance of 3 to 1 in the former and 10-15 to 1 in the latter⁴⁵. In our study, only male transgenic mice had the skin lesions of spindle

cell proliferation and tumour development, despite equivalent levels of *tat* mRNA expression in female mice. This male preponderance has not yet been explained but may be due to hormonal or other genetic factors⁴¹. We are presently investigating whether the sexual difference in phenotype is hormonally based. (5) Histologically, KS consists of interweaving bands of spindle cells and vascular structures which can appear as slit-like spaces or delicate capillaries³⁷⁻⁴³. The spindle cells may exhibit a wide range of nuclear pleomorphism, and in this background of proliferating spindle cells, abnormally large endothelial cells may be present in vascular structures. Our murine dermal tumours also had well defined abnormal vascular structures present among interweaving bands of spindle cells and only in limited areas were the endothelial cells clearly neoplastic. Frequently, the early lesions consisted of poorly defined aggregates of spindle-like cells which progressed to interweaving fascicles of spindle cells, scattered erythrocytes, nuclear pleomorphism, and mitotic figures.

In general, the similarities between the murine dermal lesions and human KS are remarkable, particularly in view of the fact that KS-like lesions are not seen in mice. In fact, no skin lesions were seen in a two-year survey of 1,000 CD1 mice looking at the incidence of spontaneously arising neoplasms⁴⁶. Although controversy still surrounds the cell of origin of KS, it is generally considered to be derived from endothelial cells, either of vascular or lymphatic origin^{39,47}. We are initiating studies with cell markers to determine the different cell types present in our murine dermal lesions.

Discussion

Given the occurrence of Kaposi's sarcoma in different populations, its aetiology is probably multifactorial in nature. A variety of environmental causes have been proposed^{10,48}. An increased incidence of KS has also been noted in immunocompromised patients, and it is possible that an immunocompromised state may exacerbate the effects of infectious agents such as cytomegalovirus⁴⁹. Although both the immunodeficiency state and cytomegalovirus infection frequently accompany HIV infection⁴⁸, Kaposi's sarcoma is also seen in HIV-infected individuals who do not have measurable immunodeficiency or abnormalities of immune regulation⁴⁰.

Because our male transgenic mice developed dermal lesions resembling KS, we would propose that HIV, and specifically the *tat* gene product, contributes to the development of KS. HIV is present in the skin, as shown by studies detecting both viral core protein and viral particles in epidermal Langerhan's cells, which may be a significant reservoir of HIV in the body³⁶. Although a study analysing KS tumour biopsies for HIV DNA sequences by Southern blot analysis was negative, it has a stated sensitivity of only 0.2 viral genome equivalence per cell⁵⁰. In addition, we are not aware of any reports regarding HIV *tat* gene expression in KS lesions. *In situ* hybridization studies on our dermal lesions will help determine exactly how the effects of the *tat* gene product are mediated.

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The product of the *Drosophila* gene *vasa* is very similar to eukaryotic initiation factor-4A

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The vasa gene product of Drosophila melanogaster is required only in the female germ line. Progeny of females homozygous for vasa mutations lack posterior structures and pole cells. Isolation and characterization of vasa genomic and complementary DNA clones show that the transcript is abundant in the female germ line and early embryos only. The predicted amino acid sequence is very similar to those of the translation initiation factor eIF-4A and the human nuclear antigen p68.

BOTH maternally active and zygotically active genes are essential for the specification of anterior-posterior positional information. In the embryo of *Drosophila melanogaster*, the maternal genes can be grouped into three classes: the anterior group, with three members: *bicoid*, *exuperantia* and *swallow*¹⁻⁷; the terminal group, the *torso*, *torso-like* and *trunk* genes^{1,8}; and the posterior group which includes seven genes: *vasa*, *valois*, *staufer*, *tudor*, *oskar*, *nanos* and *pumilio*^{1,9-11}. The progeny of females homozygous for mutations at any of these loci lack components of the anterior-posterior pattern: embryos from mothers homozygous for an anterior group mutation lack head and thoracic structures, embryos from mothers homozygous for a terminal group mutation lack structures at both the extreme anterior and posterior ends and embryos from mothers homozygous for a posterior group mutation lack abdominal structures. Five of the posterior group genes (*vasa*, *valois*, *staufer*, *tudor* and *oskar*) also affect formation of the pole cells, the progenitors of the germ line¹. Embryos from females homozygous for mutations at any of these genes completely lack pole cells and polar granules, which normally begin to form in the ovary at stage 10 (stages defined by King¹²) and are essential for the formation of functional pole cells¹³.

The maternal genes are active before the start of embryonic transcription and can thus be considered to be at the top of a temporal hierarchy of genes whose expression and interactions cooperate to specify anterior-posterior positional information. The function of one posterior group gene, *vasa*, is required at a very early stage in germline development; in females homozygous for a deletion of *vasa*, germ-line development usually becomes aberrant when the oocyte differentiates (R. Lehmann, unpublished observations). Females homozygous for weaker

alleles of *vasa* develop eggs, but these are abnormal; the resulting embryos have no pole cells and show abnormal posterior development. Males homozygous for a *vasa*⁻ deficiency (or for the weaker alleles) are indistinguishable from wild-type males in their viability and fertility, indicating that the requirement for *vasa* is specific to the female germ line.

The multiple phenotypes of *vasa* mutations indicate that this gene may be essential in *Drosophila* both in oocyte formation and in the specification of the posterior structures of the embryo. Here, we report that the product of the *vasa* gene has a remarkably similar primary structure to the eukaryotic translation initiation factor eIF-4A (ref. 14) and the human nuclear antigen p68 (ref. 15). These three gene products define a new family of proteins which are conserved over a large evolutionary distance.

Isolation of the gene

The *vasa* gene had been localized cytologically to polytene chromosome divisions 35BC (ref. 1), in a genetically well-characterized region^{16,17}. The results of further mapping experiments are shown in Fig. 1. Complementation analysis of neighbouring mutations with a large number of deletions shows that *vasa* is located between two genes defined by recessive lethal mutations, *l(2)35Cf* and *l(2)35Cb*. Of immediate interest is the deletion mutation *Df(2L)A267* which does not complement *vasa* but does complement *l(2)35Cb*. The distal end of *Df(2L)A267* is between the *no ocelli* (*noc*) and *outspread* (*osp*) genes, within the cloned region adjacent to *Adh* (ref. 18).

A genomic library was constructed in the EMBL4 phage vector¹⁹ from *Df(2L)A267/CyO* flies and a clone, v1.04, which spans the deficiency breakpoint, was isolated. Starting from here, a 53-kilobase (kb) chromosomal walk was completed

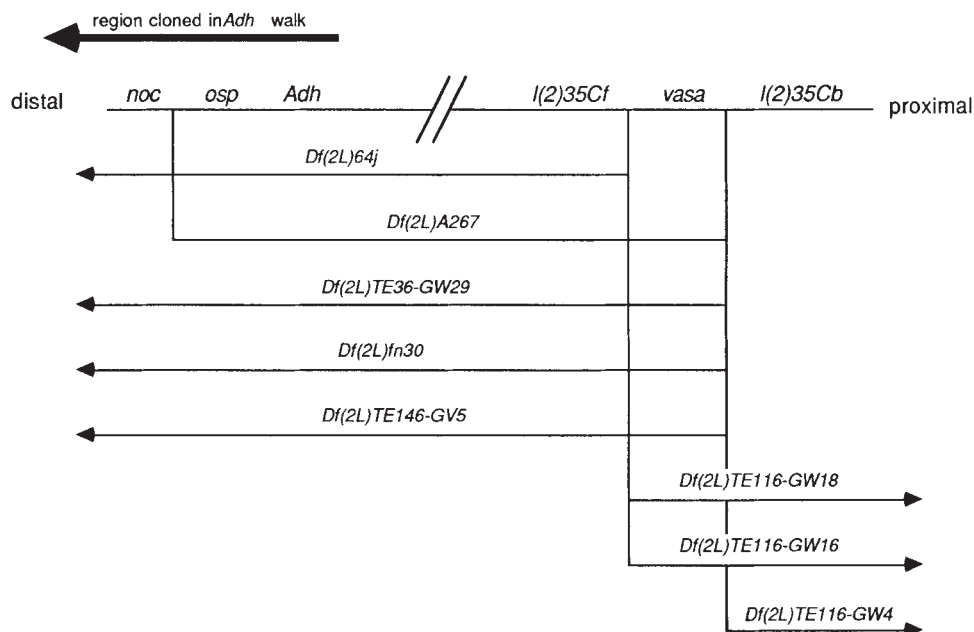


Fig. 1 Genetic map of the *vasa* region. All of the deficiencies have been described elsewhere (refs 16, 17, 37, 38); the mapping of the *Df(2L)A267* distal breakpoint to the *Adh* chromosomal walk was as described elsewhere¹⁸.

(Fig. 2a). The presence of sequences in v1.04 adjacent to both deficiency breakpoints was verified by *in situ* hybridization to polytene chromosomes (Fig. 2b). The walk was long enough to cross the breakpoints of both the deficiencies which limit the region essential for wild-type *vasa* function: *Df(2L)64j*, which deletes only sequences distal to *vasa*, maps 13.5–17.6 kb distal to the zero point (defined as the *EcoRI* site immediately proximal to the starting point), and *Df(2L)TE116-GW4*, which deletes only sequences proximal to *vasa* and maps 15.5–18.5 kb proximal to the zero point. Thus the entire chromosomal segment essential for wild-type function of *vasa* is included in the 29–36 kb of DNA between these two breakpoints. Moreover, the breakpoints of those seven known deficiencies whose genetic breakpoints have been mapped between *vasa* and *l(2)35Cf*, or between *vasa* and *l(2)35Cb*, have been located on the chromosomal walk (Fig. 2c). Crosses to generate flies with the *Df(2L)TE36-GW29*, *Df(2L)A267*, *Df(2L)fn30* or *Df(2L)TE146-GV5* chromosomes heterozygous with either the *Df(2L)TE116-GW18* or *Df(2L)TE116-GW16* chromosomes were carried out. All of these combinations were viable (for example *TE116-GW18/A267*, 199/748; *TE116-GW18/TE146-GV5*, 46/159; *TE116-GW18/TE36-GW29*, 23/136; and *TE116-GW18/fn30*, 72/438; expectation 33%) and male-fertile. Their only phenotype was that female germ-line developments topped early characteristic of a total lack of *vasa* function. Thus, of the 29–36 kb of DNA which could include the *vasa* gene, the homozygous deletion of 19.5–24 kb (as in *TE146-GV5/TE116-GW18* flies) affects only *vasa* function.

The *vasa* transcript

Both *Df(2L)TE36-GW29* and *Df(2L)TE116-GW18* break in the same genomic 4.6-kb *EcoRI* fragment. This genotype is deleted for 2–4 kb only of genomic DNA, but the *trans*-heterozygous females exhibit the null *vasa* phenotype. We used this genomic *EcoRI* fragment as a probe for potential *vasa* transcripts. An abundant 2.0-kb RNA hybridized to this probe. This transcript is present in adult females but not in males. It is present in the ovaries of adult females, but is not detectable in the remaining somatic tissue (Fig. 3). In a more detailed study of its distribution, we found that the 2.0-kb RNA is abundant only in adult females and in 0–3-h-old embryos, although a basal constitutive level of about 1% of the adult expression level is present in all developmental stages. We believe that this 2.0-kb transcript is the major product of the *vasa* gene. We also find two minor transcripts: one of about 4 kb which seems to be regulated

temporally in a similar way as the major transcript and another of approximately 3 kb which is present at low levels throughout development but is slightly more abundant in 6–24-h-old embryos. This 3-kb transcript is only detected with the 4.6-kb *EcoRI* fragment; the same filters probed with restriction fragments adjacent to those which hybridize to the 2.0-kb and 4-kb transcripts do not reveal the 3-kb transcript. The properties of the 4-kb transcript are consistent with it being an alternative splicing form of the 2.0-kb transcript, whereas the 3-kb transcript may originate from another, cross-hybridizing locus. We have not investigated these minor transcripts further.

The major 2.0-kb transcript is complementary to sequences included in the 4.6-kb *EcoRI* fragment extending from coordinates –6.2 to –1.6, the 1.6-kb *EcoRI* fragment extending from –1.6 to 0 and the 0.6-kb *EcoRI* fragment extending from –6.8 to –6.2 kb, but not to the *SalI* fragment extending from –5.2 to –3.4. The localization of this transcript is consistent with that predicted for the *vasa* transcript by the mapping of the various deficiency breakpoints. Moreover, no transcripts are detectable (even on exposures 10–20 times longer than those in Fig. 3) when the filters are probed with any other sequence from the region between the *Df(2L)64j* and the *Df(2L)TE116-GW4* breakpoints (data not shown), indicating that the transcripts described are indeed the only possible products of the *vasa* gene. RNA was prepared from adult females homozygous for seven different mutant *vasa* alleles. Four of these had similar amounts of the 2.0-kb transcript as the wild-type. The other three, which are the strongest alleles in terms of their posterior-group phenotype, have no detectable 2.0-kb transcript (data not shown).

Localization of the *vasa* transcript

The localization of the *vasa* transcript in ovaries and early embryos was studied by *in situ* hybridization to tissue RNA using the 4.6-kb *EcoRI* fragment as a probe (Fig. 4). The transcript is evident in the earliest stages of oogenesis (Fig. 4a), which would be predicted from the phenotype of females deleted for both copies of *vasa*, which usually arrest oogenesis before oocyte differentiation (at about stage six). By stage 10, the *vasa* transcript is fairly abundant in the nurse cells and is exported to the oocyte; there is no labelling in the somatic follicle cells indicating that the transcript is germ-line specific. Moderate and uniform labelling continued in oocytes and nurse cells of later stages (Fig. 4b). Early cleavage embryos also label moderately and uniformly, but the intensity of label decreases fairly rapidly

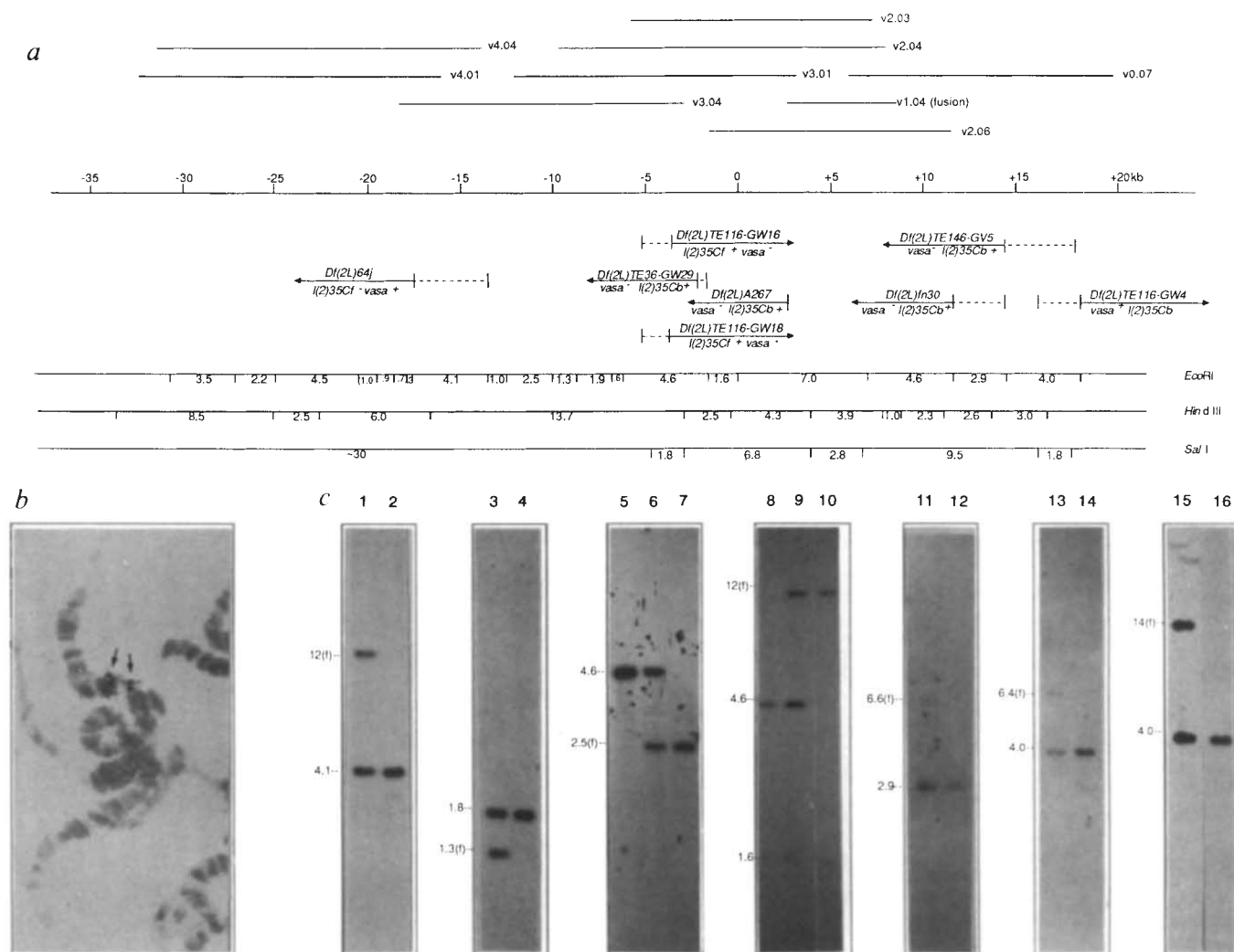


Fig. 2 *a*, Molecular map of the *vasa* region, with deficiency breakpoints which fall within the chromosomal walk. Broken lines represent the maximum limits within which the breakpoints must lie. *b*, Salivary gland polytene chromosomes from Canton-S wild-type larvae probed with ³H-labelled v1.04 DNA, showing two sites of hybridization corresponding to the sequences adjacent to *noc* and *vasa* (arrows). *c*, Restriction-digested genomic DNA electrophoresed, blotted and probed with ³²P-labelled genomic restriction fragments, show deficiency breakpoint localization. The numbers are the sizes in kb of the Canton-S wild-type restriction fragments, the numbers marked (f) are the sizes in kb of the novel fusion fragments. *Df(2L)A72* is a deficiency that removes the entire region included in the chromosomal walk¹⁸ and is included as a control for fragment length polymorphisms in the *CyO* balancer chromosome. *Df(2L)64j/CyO* (lane 1) and *Df(2L)A72/CyO* (lane 2) DNA was cut with *EcoRI* and probed with the 4.1-kb *EcoRI* fragment from v3.04. *Df(2L)TE116-GW16/CyO* (lane 3) and *Df(2L)A72/CyO* (lane 4) DNA was cut with *SalI* and probed with the 1.8-kb *SalI* fragment from v2.04. *Df(2L)A72/CyO* (lane 5), *Df(2L)TE116-GW18/CyO* (lane 6) and *Df(2L)TE116-GW18/Df(2L)TE36-GW29* (lane 7) DNA was cut with *EcoRI* and probed with the 4.6-kb *EcoRI* fragment from v2.04. *Df(2L)A72/CyO* (lane 8), *Df(2L)TE36-GW29/CyO* (lane 9) and *Df(2L)TE36-GW29/Df(2L)TE116-GW18* (lane 10) DNA was cut with *EcoRI* and probed with the 2.5-kb *HindIII* fragment from v2.04. *Df(2L)fn30/CyO* (lane 11) and *Df(2L)A72/CyO* (lane 12) was cut with *EcoRI* and probed with the 2.9-kb *HindIII* fragment from v0.07. *Df(2L)TE146GV5/CyO* (lane 13) and *Df(2L)A72/CyO* (lane 14) DNA was cut with *EcoRI* and probed with the 4.0-kb *EcoRI* fragment from v0.07. *Df(2L)TE116-GW4/CyO* (lane 15) and *Df(2L)A72/CyO* (lane 16) DNA was cut with *EcoRI* and probed with the 4.0-kb *EcoRI* fragment from v0.07. ³H-labelled DNA was prepared by nick translation as described by Pardue³⁹ and restriction fragments were labelled in low-melting-point agarose^{40,41}.

with increasing developmental age: syncytial blastoderm embryos label weakly; by the cellular blastoderm stage there is no detectable signal above background levels (Fig. 4c). There is no spatial asymmetry in the disappearance of the signal, nor is there any localization of transcripts to the pole cells (Fig. 4d).

Structure of the gene

We screened cDNA libraries made from ovaries and from 0–4-h-old embryos for clones homologous to the transcribed region⁴. Three clones were recovered and shown to be overlapping by restriction analysis. We determined the nucleotide sequence of the longest of these, which had 2,013 base pairs (bp). The clone, cv1.092, hybridizes to the same genomic fragments as the major transcript and contains a translation start site with a *Drosophila* consensus flanking sequence²⁰ at nucleotide 15 of the cDNA.

Genomic and cDNA sequencing gives an open reading frame of 660 amino acids to a termination codon. We believe that cv1.092 corresponds to a nearly full-length copy of the 2.0-kb *vasa* transcript, containing the entire protein coding region. Comparison of the sequence of the cDNA with the corresponding genomic DNA sequence shows that the *vasa* gene has a relatively complex organization, with six introns (Fig. 5a). Five of these are rather small (36–70 nucleotides), but the second is approximately 3,700 nucleotides in length. All of the splice junctions fit the *Drosophila* consensus²¹.

The nucleotide and predicted amino acid sequences are listed in Fig. 5b. The predicted amino acid sequence is of a protein with a relative molecular mass of 72,324. A search of the SWISS-PROT database using the FASTP computer program²² indicated that there was 29.1% amino acid identity between the *vasa*

Fig. 3 RNA prepared from various sources, electrophoresed through formaldehyde-agarose gels, blotted as described⁴² and probed with the 4.6-kb *Eco*RI fragment from v2.04 labelled with ³²P. Selection of poly(A)⁺ RNA was carried out on Hybond-mAP paper (Amersham), according to the manufacturer's instructions. Total (20 µg; lanes 1 and 2) and poly(A)⁺ (5 µg; lanes 3 and 4) RNA prepared from adult males (lane 1 and 3) and females (lanes 2 and 4). Total RNA taken from dissected ovaries (three pairs, lane 5) and from the remainder of the female adult (three flies, lane 6). The band which migrated slowly on the gel was genomic DNA, which was not removed from these preparations. Total RNA taken from dissected ovaries (three pairs, lane 7) and from dissected testes (20 pairs, lane 8). The amount of RNA loaded in lanes 5 and 6 and in lanes 7 and 8, was approximately equal, as measured by hybridization to sequences codin for cytoplasmic actin (act-A2, ref. 43). Poly(A)⁺ RNA (5 µg) prepared from 0–1.5-h-old embryos (lane 9), 1.5–3-h-old embryos (lane 10), 3–4.5-h-old embryos (lane 11) and 4.5–6-h-old embryos (lane 12). Poly(A)⁺ RNA (5 µg) prepared from 0–6-h-old embryos (lane 13), 6–12-h-old embryos (lane 14), 12–24-h-old embryos (lane 15), first instar larvae (lane 16), second instar larvae (lane 17), third instar larvae (lane 18), early pupae (1–3-days-old; lane 19); late pupae (3–5-days-old; lane 20) and adults (lane 21).

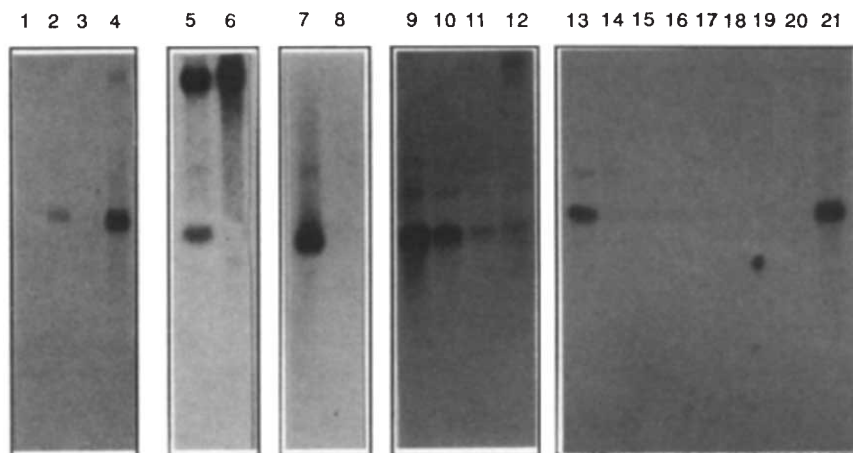
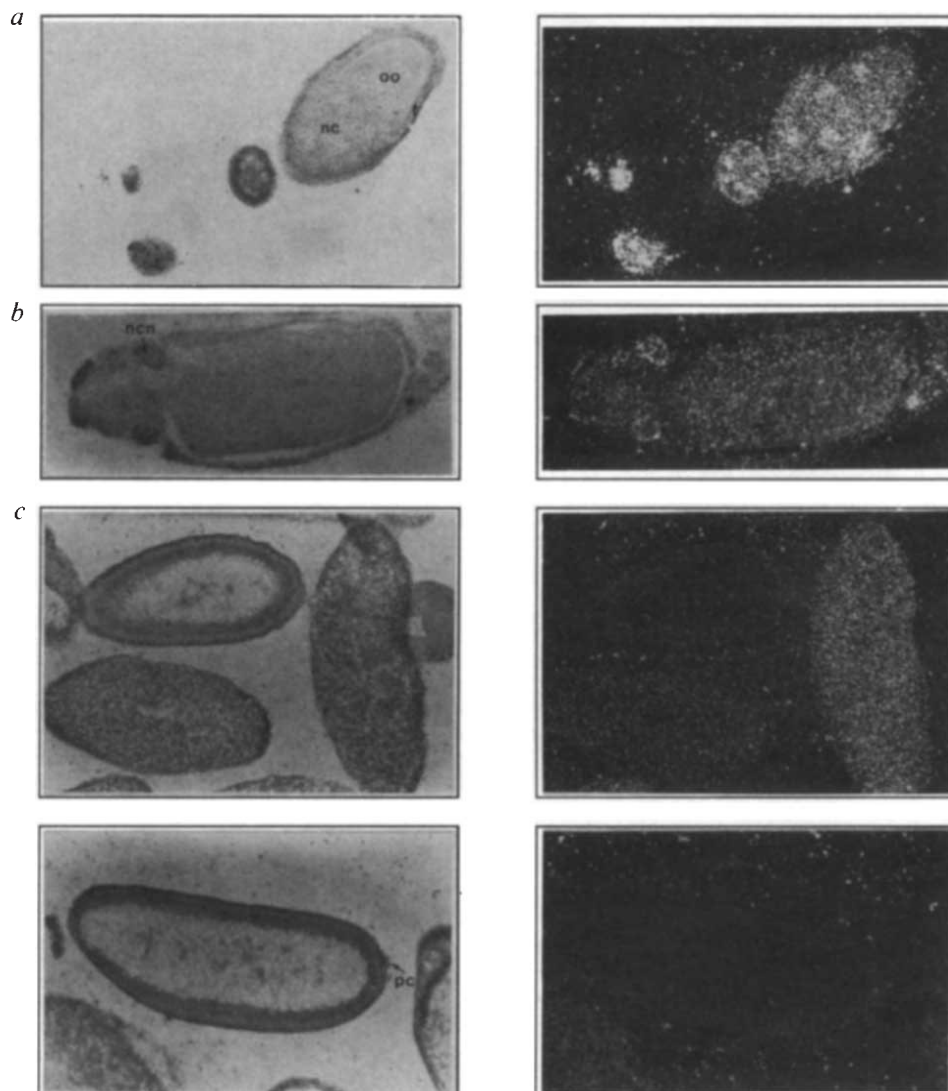


Fig. 4 *a*, Left to right: stage 2, stage 6 and stage 10a egg chambers; nc, nurse cells, oo, oocyte, f, follicle cells. *b*, Stage 11 egg chamber; nc, nurse cell nucleus. *c*, Clockwise from the right: an early cleavage embryo (stage one or two), an early syncytial blastoderm embryo (stage four), a cellular blastoderm embryo (stage five). *d*, Stage five cellular blastoderm with pole cells (pc) evident. Tissue sections probed with the 4.6-kb *Eco*RI fragment from v2.04 labelled with ³⁵S by the method of Akam and Martinez-Arias⁴⁴, as modified by Sánchez-Herrero and Crosby⁴⁵. Ovaries were hand-dissected, fixed in 4% paraformaldehyde in phosphate-buffered saline, embedded first in low-melting-point agarose, and then in paraffin wax as described by Ingham *et al.*⁴⁶ Embryos were fixed and embedded as described⁴⁶. Section thickness was 8 µm. Tissue was stained with toluidine blue. Oogenesis stages are those of King¹² and embryogenesis stages are those of Campos-Ortega and Hartenstein⁴⁷.



polypeptide and murine eukaryotic initiation factor-4A (eIF-4A) over a 375-residue overlap which includes the eIF-4A ATP-binding site¹⁴. The amino acid similarity is even greater between the *vasa* product and the human nuclear antigen p68 (ref. 15). In the three-way alignment shown in Fig. 6, 105/398 (26.4%) amino acid identities are found between *vasa* and eIF-4A and

144/398 (36.2%) in the same region between *vasa* and p68. An additional 36 amino acids (9.1%) in *vasa* and eIF-4A are related by the following five conservative changes: isoleucine/valine; aspartic acid/glutamic acid; asparagine/glutamine; arginine/lysine; or serine/threonine. Twenty amino acids in *vasa* and p68 (5.1%) are related in this way. The similarity is mostly limited

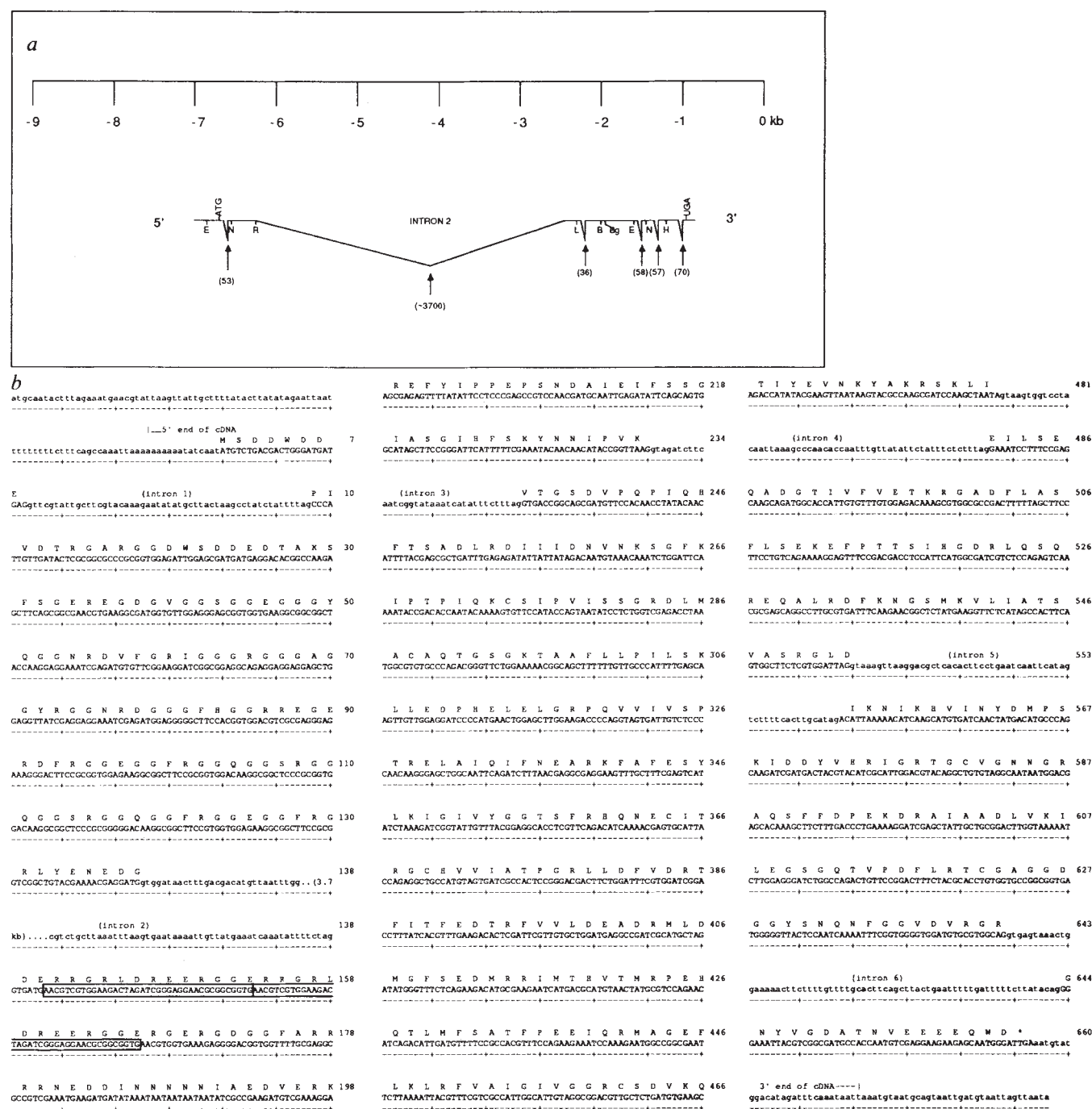


Fig. 5 *a*, Structure of the *vasa* gene. The coordinates (kb) are on the same scale as those in Fig. 2, the restriction sites listed are those used for construction of subclones for sequence determination. *B*—*Bam*HI, *Bg*—*Bgl*II, *E*—*Eco*RI, *H*—*Hind*III, *L*—*Lsp*I, *N*—*Nar*I and *R*—*Rsa*I. *b*, Genomic and cDNA sequence of the *vasa* gene. Subclones were made in the M13 vectors mp18 and mp19 (ref. 48) and sequenced by the dideoxynucleotide method⁴⁹ using Sequenase (United States Biochemical Corporation) according to the manufacturer's instructions. The cDNA was sequenced on both strands. All DNA except the interior of intron two has also been sequenced in genomic DNA. The sequence extending from the last nucleotide of codon 89 through the second nucleotide of codon 131 is made up of six copies of a very similar 21-bp motif. Two tandem copies of a 39-bp repeat are boxed; both are present in the genomic clone, but only one copy exists in the cDNA clone. Three codons differ between the genomic sequence shown and the cDNA clone: in the cDNA, codon 195 is GCC (Ala), codon 265 is TAC (Tyr), and codon 324 is GTA (Val). Sequence analysis was carried out using the University of Wisconsin Genetics Computing Group package⁵⁰.

to that portion of the peptides which is also similar to eIF-4A, although there is some similarity between the *vasa* and p68 proteins outside this region (Fig. 6); both proteins are rich in glycine near their termini. There are tracts of highly conserved residues within the similar regions. For example, the sequence VLDEADRLDMGF beginning in the putative *vasa* product at amino acid 397 is identical in p68 and conserved in 10/13 cases in eIF-4A. The type-A consensus ATP-binding site^{14,15,23,24} is also highly conserved in *vasa*, beginning at residue 287 (9/9

consensus residues; 11/17 overall identity with p68, 10/17 with eIF-4A). Other highly conserved regions include the invariant PTRELA beginning at residue 326 in the *vasa* sequence, the region surrounding and including the invariant TPGR beginning at residue 375, the invariant VINYP at residue 560, and the Y(I/V)HRIGR from residue 572 in the *vasa* protein. Some of these homologies have been previously identified in a superfamily of nucleic acid helicases^{25,26}: motif I, identified by Hodgman²⁵, is the type-A ATP-binding site (residues 287–300), motif

Ia (ref. 27) extends from residues 322–338 and motif II is found between residues 396–406. However, the more carboxy-terminal homologies have not been identified for the entire superfamily and we believe they are characteristic of a smaller, more specific, group of putative helicase proteins, of which *vasa*, eIF-4A and p68 are the first three members. We are aware of another four proteins, from *Escherichia coli*, *Saccharomyces cerevisiae*, and *Mus musculus*, that are also members of this family (P. Linder *et al.*, in preparation).

Discussion

The gene *vasa* seems to have several roles in oogenesis and in the determination of anterior-posterior positional information in the *Drosophila* embryo. The null *vasa*⁻ phenotype is the early cessation of egg development and, consistent with this, the start of *vasa* transcription is in the earliest stages of oogenesis, whereas for *bicoid*, a maternal-effect gene with no mutant phenotype that affects egg formation, transcription only begins at about stage six (refs 5, 12). Unlike the *bicoid* transcript, the *vasa* transcript is not localized within the female germ line. If *vasa* acts in a spatially asymmetric manner, as might be expected from its posterior group mutant phenotype, then the localization of the *vasa* product must occur at the protein level.

Recently, Hay *et al.*²⁸ have characterized a monoclonal antibody (Mab46F11) that recognises an antigen of relative molecular mass similar to that expected for the *vasa* product. Moreover, this antigen is expressed very early in oogenesis and accumulates at the posterior pole of the oocyte. In the early embryo this antigen is sequestered into the pole cells. Embryos from females homozygous for the mutant allele *vasa*^{PD23} (ref. 1) lack this antigen. However, this monoclonal antibody recognizes an antigen, of similar relative molecular mass, in the male germ line and in late embryos and larvae, which would be unexpected for the product of the transcripts we have identified for *vasa*. As much of the *vasa* peptide sequence is highly conserved with other proteins, which presumably have their homologues in *Drosophila*, we cannot rule out the possibility that Mab46F11 recognizes the *vasa* product along with other proteins in its family.

The marked sequence homologies between *vasa*, eIF-4A and p68 suggest that the putative *vasa* protein may be an ATP-dependent nucleic acid helicase. It may have a role in the regulation of expression of a specific set of genes at the transcriptional or translational level. The biological function of p68 is uncertain. It was first investigated because of a shared antigenic determinant with simian virus 40 large T antigen²⁹, but that determinant is located outside the region of p68 conserved in eIF-4A and *vasa*¹⁵. p68 is localized predominantly in cell nuclei¹⁵.

eIF-4A functions as part of a protein complex with two other polypeptides, eIF-4B and eIF-4F, to recognise the messenger RNA cap structure and catalyse RNA unwinding³⁰. It is a multifunctional protein^{30,31} and, in both mammals and wheat germ, is an ATP-dependent single-stranded RNA binding protein^{32,33}. There is also evidence that eIF-4A is present in the complex which scans the message for the AUG initiation codon, as molecules of eIF-4A in excess of those comprising the cap-binding complex are required for initiation of translation^{34,35}.

A complete understanding of the molecular function of the *vasa* gene must await study of its protein product. It is clear from the mutant phenotype that *vasa* cannot be the *Drosophila* homologue of eIF-4A or any other global regulator of gene expression, as it is completely dispensable for viability and fertility in males. However, aspects of the *vasa*⁻ phenotype are consistent with its product being a similar protein to eIF-4A, but which is active only on a set of genes involved in oocyte differentiation and specification of posterior and polar information. For instance, the pleiotropic nature of *vasa*⁻ mutations, involving multiple processes in oocyte development, clearly indicates that the gene has a regulatory role. Recently, a second

Vasa.Pep	MSDDWDDEPI	VDTRGARGGD	WSDDDED TAKS	FSGEREGDGV	GGSGGEGGGY
Vasa.Pep	QGGNRDVFR	IGGGRGGGAG	GYRGNRRDGG	GFHGRRREGE	RDRFGGEGGF
P68.Pep					F
Vasa.Pep	RGGQGGSRGG	QGGSRGGGQG	FRGGEGGFRG	RLYENEDGDE	RRRLDREER
P68.Pep	HSQFGGSRAG	PLSGK....K	FGNPGKLVK	KKWNLDLPK	FEKNFYQEHF
Vasa.Pep	GGERRRGLRD	EERGGGERGER	GDGGFARRRR	NEDDINNINN	IAEDVERKRE
P68.Pep	DLARRTAQEV	ETYRRSK...			
Vasa.Pep	FYIPPEPSND	AIEIFSSGIA	SGIHFSKYNN	IPVKVTGSDV	PQPIQHFTSA
Eif4a.Pep			M	EPEGVIESNW	NEIVDSFDDM
P68.Pep				EITVRGHNC	PKPVLNFYEA
Identities				---V---	---F---
Vasa.Pep	DLRDIIDNV	NKSGFKIPTP	IQKCSIPVIS	SGRDLMACAQ	TGSGKTAFL
Eif4a.Pep	NLSESLRGI	YAYGFEKPSA	IQQRAILPCI	KGYDVIAQAQ	SGTGKATFA
P68.Pep	NFPANVMVDI	ARQNFTPEPTA	IQAQGWVVAL	SGLDVMGVQA	TGSGKTLISYL
Identities	-----	---F--P--	IQ-----	-G-D--AQ	-G-GKT---
Vasa.Pep	LPILSKLLED	PHELELGRPO	VVIVSPTRER	AIQIFNEARK	FAPESYLKIG
Eif4a.Pep	ISILQOI...	..ELDLKATQ	ALVLAPTRER	AQQIQKVVMA	LGDYMGASCH
P68.Pep	LPAIVHINHQ	PFLERGDGPI	CLVLAPTRER	AQQVQVAAE	YCRACRLKST
Identities	-----	-----	---PTREL	A-Q-----	-----
Vasa.Pep	IVYGGTSFRH	QNECITRGC.	HVVIATPGRL	LDFVDRTPIT	FEDTRFVULD
Eif4a.Pep	ACIGGTVNRA	EVQKLQMEAP	HIIVGTPEGR	FDMLNRRYLS	PKYIKMFVLD
P68.Pep	CYGGAPKPG	QIRDLERGG	ECIATPGRL	IDFLECGKTN	LRRTTYLVLD
Identities	---GG---	-----	---TPGR-	-D-----	-----VLD
Vasa.Pep	EADRLMDMGF	SEDMRRIMTH	VTRMPEHQTL	MFSATFPPEI	QRMAGEFLKL
Eif4a.Pep	EADMLSRGF	KDQIYDIPQ.	.KLNSNTQVV	LLSATMPSDV	LEVTKKFMRD
P68.Pep	EADRLMDMGF	EPQIRKIVD.	.QIRPRDQTL	MWSATWPKEF	RQLAEDFLKD
Identities	EAD-ML--GF	-----I-	-----Q-	--SAT-P--	-----F--
Vasa.Pep	R.FVAIGIVG	GRCSDVQKTI	YEVNKYAKRS	KLIEILSE..	.QADGT..IV
Eif4a.Pep	PIRILVKKEE	LTLEGIRQFY	INVEREWEKL	DTLCDLYE..	.TLTITQAVI
P68.Pep	YIHINIGALE	LSANHNILQI	VDVCHDVEKD	EKLIRLMEEI	MSEKENKTIV
Identities	-----	-----	---V---	-----L-E-	-----
Vasa.Pep	FVETKRGADF	LASFLSEKEF	PTTSIEGDRL	QSOREQALRD	FKNGSMKVLII
Eif4a.Pep	FINTRRKVDW	LTEKMHARDF	TVSAMEGDM	QKERDVIMRE	FRSGSSRVLI
P68.Pep	FVETKRRCD	LTRKMRDGG	PAMGIEGDKS	QERDQVNLN	FKHGKAPILI
Identities	F--T-R--D-	L-----	-----EGD--	Q--R-----	F--G-----LI
Vasa.Pep	ATSVASRGLD	IKNIKHVINY	DMPSKIDDYV	BRIGRTGCVG	NNGRAQSFFD
Eif4a.Pep	TTDLLARGID	VQOVSLVINY	DLPTNRENYI	BRIGRGGRFG	RKGVAINMVT
P68.Pep	ATDVASRGLD	VEDVKFVINY	DYPNSSEDIY	BRIGRTARST	KTGTATYFTT
Identities	-T-----RG-D	-Y-----	D-P-----	BRIGR-----	--G-A-----
Vasa.Pep	PEKDRATAAD	LVKILEGSGQ	TVPDFLRTCG	AGGDDGYSNQ	NFGGVDRGR
Eif4a.Pep	EEDKRTLRLD	ETFYNTSIEE	MPLNVADLI*		
P68.Pep	PNNIKQVSDL	ISVLREANQA	INPKLLQVLE	DRSGRSRGR	GMKDDRRDR
Identities	-----	-----	-----	-----	-----
Vasa.Pep	..GNYVGDA	NVEEEQWD*			
P68.Pep	YSAGKRGGFN	TPDRRENYDR	GYSSLLKRDF	GAKTQNGVYS	AANYTNGSFG
P68.Pep	SNFVSAGIQT	SFRTGNPTGT	YQNGYDSTQQ	YGSNVPMNH	GMNQAYAYP
P68.Pep	ATAAAPMIGY	PMPTGYSQ*			

Fig. 6 Alignment of the peptide sequences of *vasa*, eIF-4A and p68. Identical residues in all three proteins are listed in the fourth row, residues identical in at least two of the three proteins are in bold type. The sequences are complete as reported; the p68 sequence is lacking some residues from the amino-terminal end¹⁵.

*Indicates the carboxy-terminal end of the protein.

murine eIF-4A gene has been reported³⁶, whose expression is localized to a different set of tissues from the first, perhaps providing a precedent for specific gene regulation at the translational level by this factor. It is also interesting that the posterior group of maternal genes has more members than either of the other two groups, perhaps indicating that a relatively more complex set of regulatory interactions occurs among those genes.

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to P.L. Sequence data will appear in the EMBL/Genbank/DBJ Nucleotide Sequence Databases with the accession numbers X12945 and X12946.

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LETTERS TO NATURE

Lensless microwave imaging using the Hartley transform

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We recently reported a method of optical phase measurement¹ that involves the creation of a Hartley transform² plane, which is constructed by the superposition of two Fourier transforms³, one of which was rotated spatially by 180° and shifted in phase by 90°. Because the Hartley transform, unlike the Fourier transform, is entirely real, it is able to extract more information when a phase-insensitive detector (for example, an optical detector) is used. In particular, the Hartley intensity encodes the location of the centroid of a source, whereas the intensity distribution of a Fraunhofer diffraction pattern does not change when the source shifts because source location is hidden in the phase information. Through the Hartley transform one gains access to the Fourier phase by amplitude measurements only. We have now extended this technique to the microwave range, and have also demonstrated how to obtain an image of an isophase source without using a phase-sensitive detector.

Because the wavelength we used (27.87 mm, corresponding to a frequency of 10.75 GHz) is ~44,000 times greater than optical wavelengths, the arrangement of the apparatus is necessarily different. For image rotation a corner reflector is used instead of a lens and, in addition, it is possible to dispense with the Fourier-transforming lens by working in the far field of the source. Here we merely demonstrate the feasibility of the technique and do not aim to describe a fully engineered instrument. For this purpose, image reversal rather than image rotation suffices, and records whose length is limited by available equipment are accepted, as they are sufficient to demonstrate the experimental principle. Not only has the phase of a microwave signal been determined by intensity measurements alone but also an image has been obtained of an isophase source, albeit to a resolution limited by the length of the record.

The Hartley transform of a real source function $f(x)$ is given by

$$H(u) = \int_{-\infty}^{+\infty} f(x) \operatorname{cas}(2\pi ux) dx$$

where $\operatorname{cas}(\theta) = \cos(\theta) + \sin(\theta)$. The Fourier transform, by contrast, is found from

$$F(u) = \int_{-\infty}^{+\infty} f(x) e^{-2\pi iux} dx$$

In general, the Fourier transform $F(u)$ will be complex because of the complex nature of the kernel, whereas the cas function that serves as the kernel for the Hartley transform is real, so that the transform $H(u)$ will also be real. The Fourier phase ϕ , which is normally thought of as an angle in the complex plane, can be recovered directly from the Hartley transform through $\tan(\phi + \pi/4) = H(-u)/H(u)$; the same information is conveyed by the Hartley phase ϕ_H , defined by $\tan(\phi_H) = H(-u)/H(u)$.

The Hartley transform can alternatively be expressed as the sum of two Fourier transforms of the source:

$$H(u) = [F(u) + \exp(-i\pi/2)F(-u)] \times \exp(i\pi/4)/\sqrt{2}$$

The complex factor $\exp(i\pi/4)$ is a consequence of the phase difference of $\pi/4$ between the Hartley kernel and the real part of the Fourier kernel; it has no significant effect on the character of $H(u)$. At optical wavelengths each of the Fourier transforms above can be obtained using a lens, but for microwaves, for which aperture sizes are usually much smaller relative to the wavelength λ , the Fourier transform can also conveniently be obtained from the far-field Fraunhofer diffraction pattern.

Figure 1 shows the interferometer used to perform a one-dimensional Hartley transform by the method of superimposing two Fourier transforms of the source. The source distribution $f(x-d)$ is a 67-mm-wide horn, T, transmitting with a frequency of 10.75 GHz. The horn can be moved along the x -axis to give different shifts d from the origin O. Part of the radiation incident on the beamsplitter B is transmitted, reflects off mirror M1 and is then redirected by the beamsplitter to the output domain (labelled x') to furnish the Fourier transform $F(u)$. The beamsplitter is a sheet of acrylic resin 5.5 mm thick and is discussed

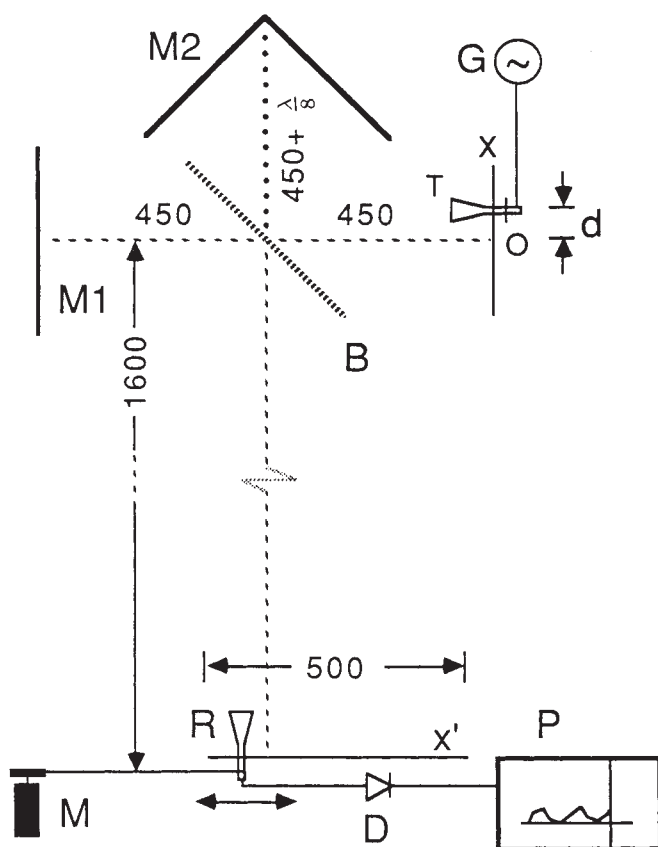


Fig. 1 Experimental arrangement used to perform the one-dimensional Hartley transform. A movable transmitting horn T in the source domain (x -axis) is used to test for appearance of the Hartley transform at the output (x' -axis). Radiation from a Gunn diode source G is emitted by the transmitting horn and then split at beamsplitter B. Part of the beam reflects from mirror M1 and then is directed to the output; the other portion of the beam undergoes a reversal at corner reflector M2 prior to recombination at the output. A motor-driven receiving horn R and detector D then provide a plot P of the one-dimensional Hartley transform. The equipment is mounted on a plane metallic optical table. Dimensions are in mm.

further below. The radiation redirected at the first encounter with the beamsplitter is then reflected by corner reflector M2, which also reverses the field about the optical axis (shown as a dotted line in Fig. 1). This signal then propagates through the beamsplitter and contributes the reversed Fourier transform $F(-u)$. When the distance from the beamsplitter to the ridge of the corner reflector and back is made $\lambda/4$ longer than the round-trip distance from the beamsplitter to the plane mirror M1, the Hartley transform relationship $H(u) = [F(u) + \exp(-i\pi/2)F(-u)]$ is realized at the output. The output intensity is recorded using a crystal detector and plotter connected to a receiving horn R which is driven along the x' -axis by motor M. The unit of u is mm^{-1} ; distance in mm along the u -axis is denoted by x' (Fig. 1), where $x' = (1,600 + 3 \times 450)\lambda u = 82,010u$.

The ability of the Hartley intensity to convey Fourier phase information was demonstrated by observing the effect in the transform domain of translating the source. It is well known that the Fourier transform of a shifted source acquires a multiplicative phase factor, whose phase gradient is proportional to the extent of the shift, whereas the Fourier intensity remains unchanged. In contrast, the Hartley transform encodes the magnitude and direction of source shift as fringes with spatial frequency proportional to the amount of shift.

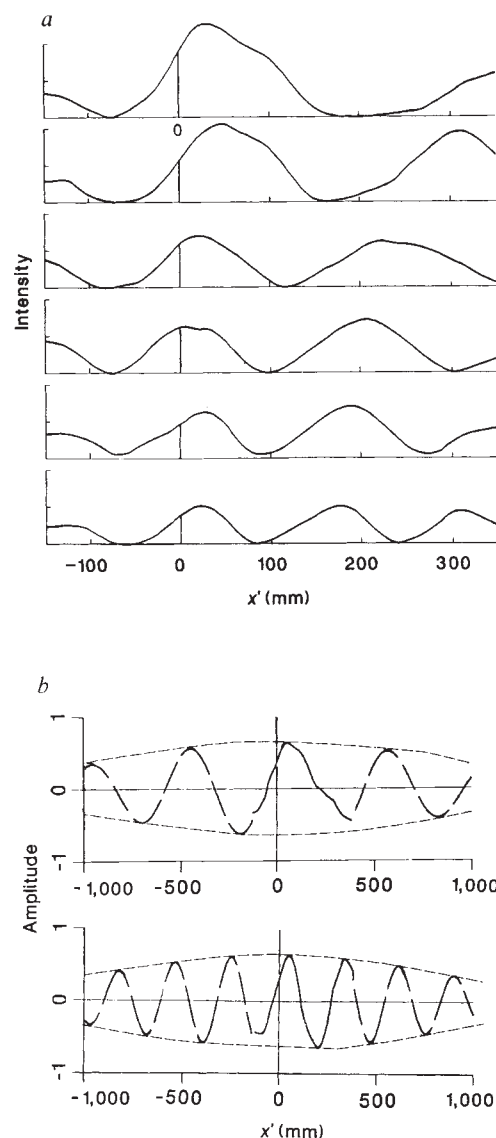


Fig. 2 *a*, The Hartley intensity measured on the x' -axis by receiving horn R for progressively larger displacements d of the transmitting horn T along the x -axis. From top to bottom: $d = 160, 185, 211, 236, 262$ and 287 mm. *b*, Superposition of the de-rectified data (full line, near centre) and the Hartley transform expected from theory (broken line). Top, $d = 160$ mm; bottom, 287 mm.

Figure 2*a* shows a series of Hartley intensity observations for progressively larger displacements of the source. The frequency of the fringes is directly proportional to the amount of displacement. Figure 2*b* illustrates, for the extreme shifts $d = 160$ and $d = 287$ mm, that the expected Hartley transform, calculated from a knowledge of the dimensions of the apparatus and wavelength (broken line to either side of $x' = 0$), is a cas function passing through $1/\sqrt{2}$ of its peak value at $u = 0$ and decaying under an envelope $\text{sinc}(wu - 0.5) + \text{sinc}(wu + 0.5)$, where w is the horn width in wavelengths and $\text{sinc}(\theta) = \sin(\pi\theta)/\pi\theta$. This envelope arises as the transform of the cosinusoidal electric field distribution in the horn. We de-rectify the data by taking the square root of the measured intensity and reversing the sign at alternate zero crossings (full line near $x' = 0$); Figure 2*b* then shows that, as planned, the apparatus is in fact performing the Hartley transformation.

By applying the inverse tangent formula above, we can determine the phase. This exhibits the linear variation expected from

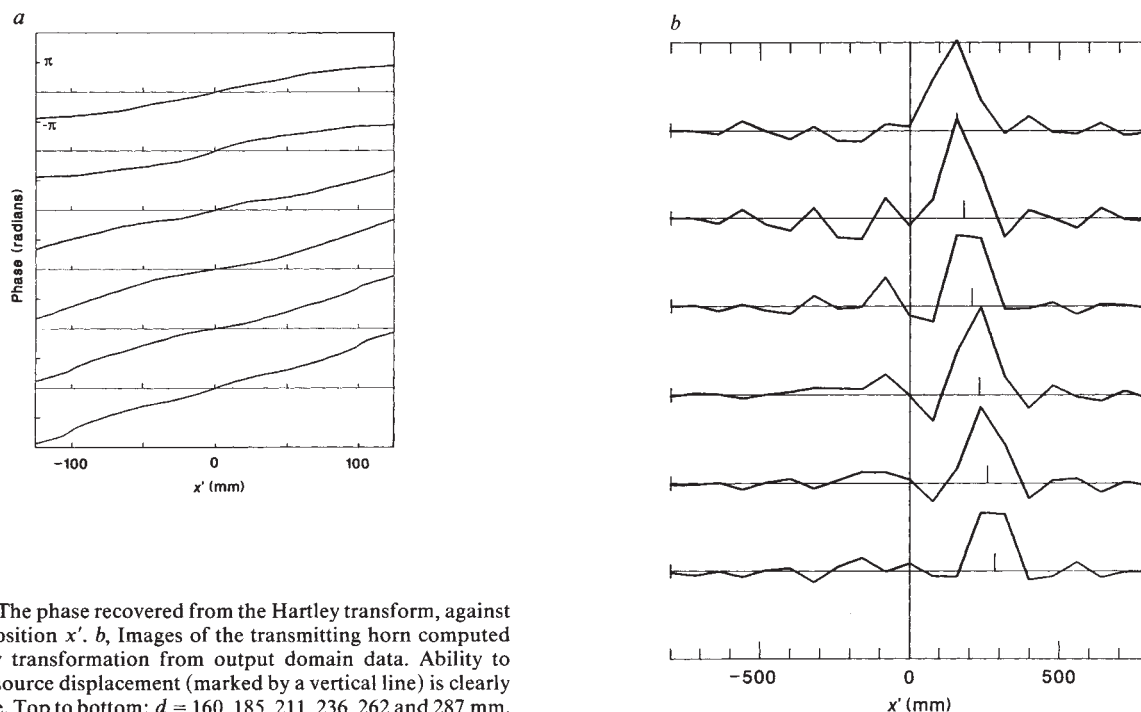


Fig. 3 *a*, The phase recovered from the Hartley transform, against detector position x' . *b*, Images of the transmitting horn computed by Hartley transformation from output domain data. Ability to detect the source displacement (marked by a vertical line) is clearly in evidence. Top to bottom: $d = 160, 211, 236, 262$ and 287 mm.

a shifted compact source, with a slope that increases according to the amount of the shift (Fig. 3*a*).

The further step may now be taken to compute an image of the source distribution from the de-rectified data. As Fig. 2*a* shows, the record lengths are restricted to -133 to 360 mm and even if the negative sides are extended artificially to equal the positive extent, creating an output record covering the range $-360 \leq x' \leq 360$ mm, not much resolution can be expected. The computation required to image the source can be considered as inversion of the Fourier transform. In practice it is more direct to invert the Hartley transformation, thus making explicit reference to phase unnecessary. The results for the six different shifts d are shown in Fig. 3*b*. The resolving power afforded by the available length of the x' -axis record is not sufficient to reveal the source shape but is clearly adequate to resolve the source shifts accurately. A more complicated distribution formed from several compact sources could certainly be imaged.

In interferometry the resolution that can be achieved in reconstruction of a source image depends only on the extent of the record made in the transform domain; in practice, however, the resolution is limited by a variety of factors. Most fundamentally, features of the source smaller in size than one wavelength will give rise only to evanescent waves which decay long before reaching the far field. Thus, even with a perfect Hartley transformer one could not expect to recover this information. Furthermore, attaining even half the limiting resolution (that is, a spatial frequency at the source of 0.5 cycles per wavelength) would require, for this instrument, a record of the transform ranging over $-1500 < x' < 1500$ mm at the output. This could be reduced by building a Hartley transformer using convergent optics instead of relying on propagation to the far field. Various configurations can be devised; the most obvious involves simply placing a lens between the beamsplitter and output domain, such that the output domain and the source domain are each at one focal length from the lens. Instead of a microwave lens, a parabolic mirror using folded optics could be used. In either case, higher spatial resolution would be obtained for a given extent of output domain.

If image rotation were performed with a lens¹ then corner reflectors could be dispensed with. Another possibility would be to perform the image rotation in one arm by means of a

parabolic mirror (rather than a lens), which would be lifted above the plane of incidence and slightly tilted.

Ideally, the reflectance and transmittance of a beamsplitter should each be 50%. In our case, the first surface reflection (reflection coefficient 0.30) combines with the second surface reflection to give an overall power reflectance of 33% rather than the ideal 50%. The superimposed beams are nevertheless equal in total power because each undergoes one reflection and one transmission; the unequal splitting only reduces the intensity, in this case by half a decibel, so the dielectric sheet is simple and effective. A beamsplitter could also consist of a grating of properly spaced thin wires parallel to the electric field. An alternative implementation in which the beamsplitter and mirrors are in the far field instead of near the source may have an application, especially if a transforming lens is used to reduce the extent of the transform plane.

Our configuration may be converted to give the Hartley transform in two dimensions by replacing the plane mirror M1 by a corner reflector oriented with its ridge parallel to the x -axis, or by replacing the corner reflector M2 by a reflecting cube corner. Alternatively, the unmodified apparatus of Fig. 1 could be used, with the field observed along the u -axis regarded as a one-dimensional slice through the full two-dimensional Hartley transform. By incrementally rotating the corner reflector or the source and measuring the corresponding one-dimensional slices at the output, tomographic techniques could be used to construct the full two-dimensional source distribution.

The results of the microwave Hartley transformer demonstrate that for those antennas that can be characterized by a real aperture distribution, the Hartley transform offers a technique for establishing the complex far-field antenna pattern and for imaging the source without the need for a phase-sensitive detector.

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An advanced selective reduction process for NO_x control

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The combustion of coal and heavy oil produces significant quantities of NO_x (NO + NO₂), which ultimately participates in photochemical smog and acid rain. Combustion modification schemes, such as staged combustion¹ and reburning², or downstream injection of selective reducing agents, particularly ammonia³ and urea⁴, can significantly reduce NO_x emissions, but their effectiveness may be limited by operating constraints. Perry and Siebers⁵ described a selective reduction process using cyanuric acid, reported to be effective over a broad temperature range. Here we report new data that indicate that the same reductions can be achieved without stainless steel to activate the reaction mechanism, if the process is combined with reburning or staged combustion (or slightly fuel-rich operation in internal combustion) to produce carefully controlled stoichiometry. The NO_x reductions that can be achieved with cyanuric acid and other —NH- and —CN-containing compounds at relatively low temperatures exceed those possible with either combustion modifications or selective reduction techniques alone or in normal combination.

NO_x is formed by high-temperature thermal fixation of atmospheric nitrogen and by oxidation of nitrogen chemically bound in the fuel. In internal-combustion engines and natural-gas flames, the dominant formation mechanism is oxygen atom attack on N₂ (ref. 6). In pulverized coal combustion, the primary source of NO_x is oxidation of nitrogen contained in heterocyclic rings within the coal⁷; in the combustion of heavy fuel oil, both mechanisms can be important⁸. Thermal NO_x emissions are most easily controlled by reducing the peak temperature using a control strategy such as flue-gas recirculation. Both thermal and fuel NO_x are susceptible to control by reducing oxygen availability, particularly during the fuel pyrolysis steps, with strategies such as staged combustion¹ and reburning². These combustion modification techniques can produce reductions from 800 to ~200 p.p.m. NO_x with coal firing, but further reductions are difficult. Downstream, selective NO_x-reduction techniques using ammonia³ or urea⁴ are very effective if the reagent can be injected at ~900–1,100 °C in fuel-lean condition, or above 1,100 °C in fuel-rich conditions^{9,10}.

Perry and Siebers⁵ reported dramatic reductions with Ar/NO/HNCO mixtures in a stainless steel flow reactor at temperatures as low as ~600 °C with —N/NO molar ratios of 1.0. They also reported similar results for diesel-engine experiments with large excesses of cyanuric acid ((HOCH)₃) decomposed above 350 °C using a stainless steel tubular reactor packed with stainless steel spheres. We conducted experiments to replicate the results of Perry and Siebers⁵ using an 8.0-cm-diameter flow reactor. Direct cyanuric acid injection was used (rather than isocyanic acid (HNCO)) because these studies were directed towards practical technological application. Reactant gases were metered with precision rotometers and exhaust concentrations were measured by chemiluminescence for NO and NO₂, NDIR for CO and CO₂, paramagnetism for O₂, and wet chemistry (with ion-specific electrodes) for HCN, HNCO and NH₃ species. HNCO was hydrolysed and measured as NH₃; before the hydrolysis any NH₃ already present was precipitated as NH₄Cl(s) using hot HCl(g). Figure 1 summarizes the results that were obtained with an initial concentration of 600 p.p.m., a reactor residence time of 1.25 s and a reactor temperature of

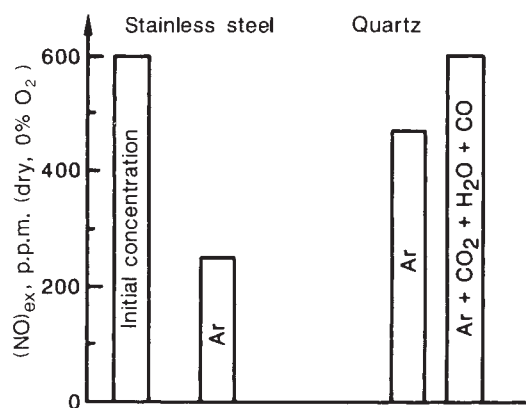


Fig. 1 Impact of reactor wall composition on reaction initiation. (650 °C, initial NO = 600 p.p.m., Ar/NO/cyanuric acid mixtures, —CN/NO = 1.5.)

650 °C. CO emissions were monitored to verify that decomposition of the cyanuric acid powder did occur. With the stainless-steel-walled reactor and Ar/NO/cyanuric acid mixture, reductions in excess of 60 % were observed with —NCO/NO = 1.5, confirming the results of ref. 5; however, when the reactor walls were lined with a quartz tube, the effective NO_x reduction decreased dramatically. Furthermore, if the argon was replaced with a mixture of 11.1 % CO₂/17.0 % H₂O/0.35 % CO and the balance of argon, which simulates combustion gases more realistically, NO_x reduction was essentially negligible at 650 °C.

These results were confirmed by testing in a 15.2-cm-diameter refractory-lined tunnel furnace fired with natural gas at ~15 kW. Dry cyanuric acid powder was added at various temperatures downstream of the main combustion zone with a molar ratio of —NCO to NO of 1.5. The initial NO concentration was 600 p.p.m. (dry, 0 % O₂ basis). We measured the amount (%) of NO remaining in the exhaust, (NO)_{ex}, as a function of the peak reaction temperature (which was ~150 °C lower than the actual injection temperature both because of the flow of N₂ (48 l min⁻¹) used to transport the dry cyanuric acid powder and because of wall heat losses.) The results (Fig. 2) indicate that under fuel-lean conditions without stainless steel present, reactant temperatures of ~1,000 °C are required to achieve significant NO_x reductions, in good agreement with temperatures reported previously for reduction with ammonia³ and urea⁴ under similar conditions. Cyanuric acid is also capable of producing dramatic NO_x reductions under fuel-rich conditions (Fig. 2) although somewhat higher temperatures are required, again consistent with experiments using ammonia⁹ and urea¹⁰.

During a test series to examine possible mechanisms for activating the low-temperature reaction chemistry without using stainless steel, we discovered that significant NO_x reductions could be achieved if cyanuric acid is used in combination with combustion modifications to provide precise control of local stoichiometry. Figure 3 summarizes a series of experiments that were conducted in the tunnel furnace⁷ with an initial NO concentration of 240 p.p.m. and a —N to NO ratio of 1.5. The solid symbols and dotted lines show results for cyanuric acid or ammonium sulphate injection under classical 'de-NO_x' conditions with 25 % excess air, and indicate an optimum reaction temperature of 1,000 °C, as has been reported previously³. Somewhat surprisingly, significantly larger reductions can be achieved over a broader temperature range if the selective reducing agent is added under slightly fuel-rich conditions (in this case the stoichiometric air/fuel ratio in the first zone, SR₁, was 0.99) and if the final burnout air is added subsequently downstream. The open symbols and solid lines in Fig. 3 represent these data, and the peak temperature refers to the temperature at which the final

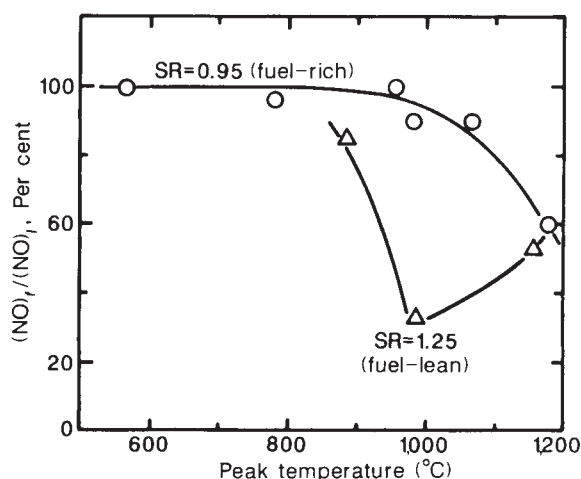


Fig. 2 Selective NO_x reduction under fuel-rich and fuel-lean conditions. (Initial $\text{NO} = 600$ p.p.m., $-\text{CN}/\text{NO} = 1.5$.)

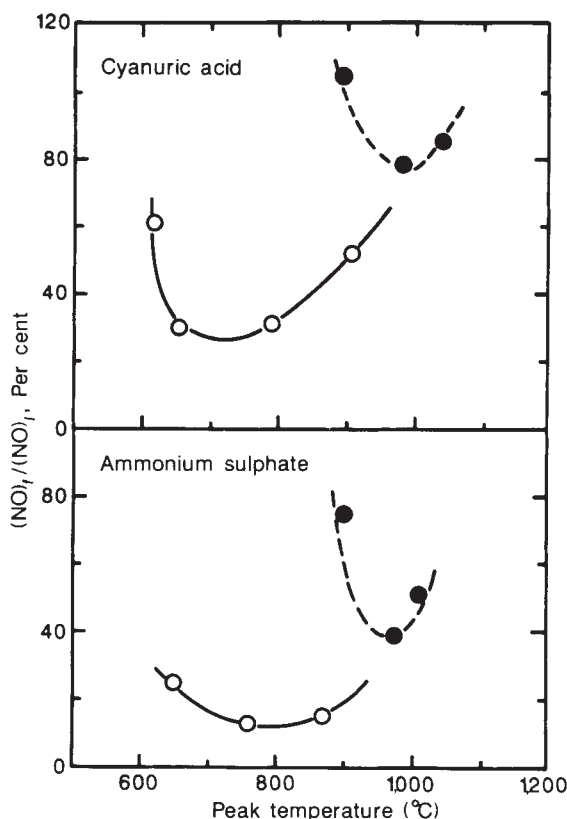


Fig. 3 Influence of injection temperature and reducing agent composition. Dotted lines, normal excess air injection; solid lines, injection in combination with staged combustion. Filled circles, SR_1 (stoichiometric air/fuel ratio) = 1.02; open circles, $\text{SR}_1 = 0.99$.

burnout air was added. The selective reducing agent was added into the fuel-rich zone at 900°C . Other compounds such as ammonium sulphate, which are significantly less expensive and potentially less toxic, can produce even larger reductions than those measured with cyanuric acid.

Figure 4 shows the results of similar testing in the tunnel furnace with an Illinois bituminous coal at a 15-kW firing rate. In these experiments, the uncontrolled NO_x emissions were $\sim 1,050$ p.p.m. Application of reburning or staged combustion

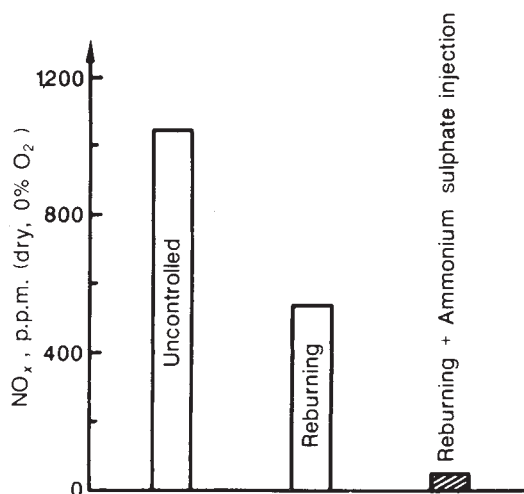
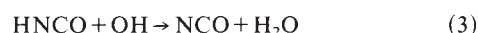
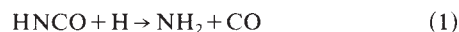


Fig. 4 Impact of combining staged combustion/reburning with selective reducing agent injection using Illinois coal. (15-kW tunnel furnace, $\text{SR}_1 = 0.9$, $-\text{N}/\text{NO} = 1.5$, 25% excess air.)

produced a 50% emission reduction. Overall emission levels of ~ 40 p.p.m. were achieved by using selective reducing agents in conjunction with the combustion modifications. In all three tests the overall excess air was 25%; in the combined technology example, the rich-zone stoichiometry was 0.9 and the $-\text{N}/\text{NO}$ ratio was 1.5. Ammonium sulphate was injected at $\sim 1,100^\circ\text{C}$ and the final burnout air was injected downstream to produce an overall excess air of 25%.

The probable mechanism of this process was investigated by use of a 97-reaction C-H-N-O-S kinetics set¹¹. The controlling reactions seem to be:



At $1,000^\circ\text{C}$, reaction (3) is the principal destruction route, which provides material for reaction (4). Reaction (4) forms N_2O as the stable product; N_2O reduction by H atoms is too slow to provide a significant conversion to N_2 under these conditions. Reactions (1) and (2) represent the global thermal de- NO_x mechanism, which yields N_2 as the predominant product.

Without a catalyst or an external radical source the overall reaction is not self-sustaining at lower temperatures. At these temperatures, however, CO oxidation can proceed by a chain-branching sequence:



and the excess radicals are available to activate reactions (1) and (2). Thus, for a low-temperature, non-catalytic process to function effectively, CO, O_2 and HNCO must all be available locally.

In summary, these results suggest that selective reducing agents can be combined with combustion modification techniques to provide NO_x reductions that are larger than those that are possible by applying the technologies simultaneously but separately. By using the stoichiometry control associated with reburning to produce a slightly fuel-rich region for selective reducing agent injection, reductions can be achieved at relatively low temperatures without the use of stainless steel or other

catalysts. In principle, this technology could have broad application to fossil-fuel-fired furnaces and boilers, stationary gas turbines and internal-combustion engines. But additional research is required to quantify the magnitude of ammonia slip and to optimize the process conditions. Preliminary exhaust measurements suggest that exit NH_3 concentrations can be as large as 10% of the added equivalent nitrogen under the low-temperature conditions with $-\text{N}/\text{NO}$ ratios of 1.5, but HNCO and HCN emissions were below detection limits (p.p.m.).

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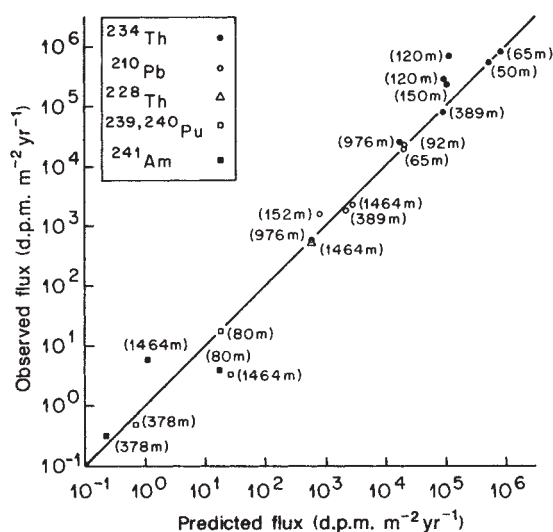


Fig. 1 Comparison of predicted (using equation (1)) and observed fluxes of five radionuclides in different water columns. Sediment trap depths (m) are shown for each sample; details given in Table 3. The solid line indicates a hypothetical perfect match of predicted and observed fluxes.

Predicting the oceanic flux of radionuclides on sinking biogenic debris

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The vertical flux of long-lived radionuclides, and of metals in general, in marine systems has been linked to the flux of particulate matter^{1,2}. Various forms of particulate matter have been shown to be capable of transporting radionuclides in the sea, with greatest interest in recent years focusing on debris of biological origin³. Here we calculate the degree to which radionuclide flux out of the euphotic zone can be attributed to sinking biodebris deriving ultimately from phytoplankton. We use experimentally determined concentration factors in phytoplankton, dissolved radionuclide concentrations in surface waters and new production estimates for specific ocean regions. Our predictions of radionuclide fluxes are generally comparable with sediment-trap measurements of radionuclide fluxes in these waters, suggesting that the downward flux from open ocean surface waters of particle-reactive radionuclides is governed principally by sinking biogenic debris.

Reports on the roles that diverse forms of biogenic debris have in transporting materials in the oceans have identified fecal pellets in particular as important vectors for transporting to depth radionuclides, metals and other elements^{1,3}. It can be shown that the flux of particle-reactive radionuclides should be directly related to zooplankton abundance⁴ and there is confirmatory evidence of this in recent studies in the Pacific⁵ and Panama Basin⁶. The flux of debris can vary seasonally and can be a function of the primary productivity in the euphotic zone^{7,8}. The scavenging rate constant of ²³⁴Th on to particles in oceanic surface waters is a function of total particle load⁹ and varies from 0.0044 d⁻¹ in oligotrophic regions to 0.18 d⁻¹ in

upwelling regions¹⁰, correlating positively with total primary productivity, and particularly with the flux of organic carbon (correlation coefficient $R = 0.988$) (ref. 10). Similarly, ²¹⁰Pb flux was recently shown to correlate strongly with organic carbon flux in the Pacific¹¹.

Our results from a sediment-trap deployment¹² at two depths in the Nares Abyssal Plain show similar strong correlations between bulk and organic fluxes and the fluxes of thorium isotopes, ²¹⁰Pb, ^{239,240}Pu and ²⁴¹Am (Table 1). Moreover, for ²²⁸Th, ^{239,240}Pu and ²⁴¹Am, the average flux at 4,832 m is similar to that at 1,464 m, because low concentrations of these radionuclides are found below 1,000 m and there can be relatively little additional scavenging on to particles sinking from 1,464 m to 4,832 m. Radionuclides such as ²¹⁰Pb and ²³⁰Th, which are produced throughout the water column from decay of ²²⁶Ra and ²³⁴U, respectively, do show additional scavenging and hence greater fluxes in the 4,832-m trap.

Because radionuclide flux may be linked to the flux of organic matter in the sea, the flux of radionuclides in marine systems could also be a function of the flux of organic carbon out of the euphotic zone, or of new production¹³. The specific function will depend on the affinity of the radionuclide in question for organic particles; the flux of an element with low particle-affinity in sea water (for example, Tc, Cs, U and Np) may show no clearly discernible correlation with new production, whereas fluxes of particle-reactive elements (for example, Th and Am) may show tight coupling with new production values. To quantify predictions of radionuclide flux in marine systems, it is therefore necessary to consider not only the new production value for a particular water column, but the degree to which the radionuclide is concentrated by the primary producers and the dissolved radionuclide concentration in surface waters. Thus, the vertical flux of radionuclides on biogenic debris sinking out of the euphotic zone in a particular water column can be expressed as

$$F = 4D C_{dw} P_n \quad (1)$$

where F is radionuclide flux (10^{-3} d.p.m. $\text{m}^{-2} \text{yr}^{-1}$), D the dissolved radionuclide concentration (d.p.m. l^{-1} of surface water), C_{dw} is the dry weight concentration factor of a radionuclide in phytoplankton (d.p.m. per kg dry weight of

Table 1 Radiochemical data for Nares Abyssal Plain sediment-trap samples 23°16.3' N, 63°55.4' W (recovered 20 September 1984)

Trap depth (m)	Collection interval (days)	Bulk flux (g m ⁻² yr ⁻¹)	Organic carbon flux (g m ⁻² yr ⁻¹)	d.p.m. m ⁻² yr ⁻¹					
				²³² Th	²³⁰ Th	²²⁸ Th	²¹⁰ Pb (×10 ⁴)	^{239,240} Pu	²⁴¹ Am
1,464	8-86	14.18	0.920	6.4±0.9	57.0±5.6	1,040±70	0.30±0.02	5.9±0.6	9.3±1.1
	86-164	8.34	0.374	8.2±1.2	28.9±2.7	437±29	0.22±0.02	2.8±0.3	5.1±1.1
	164-242	6.26	0.321	9.2±1.3	25.8±2.6	390±28	0.16±0.01	2.3±0.3	5.7±0.6
	242-320	7.68	0.478	7.2±0.7	22.2±1.4	427±17	0.18±0.02	2.9±0.3	5.3±0.7
	1-8, 320-404*	4.22	0.242	3.6±0.8	13.3±1.8	187±17	0.08±0.01	1.1±0.2	1.7±0.4
	Mean*	9.12	0.523	7.8	33.5	574	0.22	3.5	6.4
	R ₁ †			0.180	0.976	0.986	0.965	0.994	0.932
	R ₂ ‡			0.027	0.943	0.981	0.881	0.982	0.895
	8-86	11.94	0.617	18.2±1.3	96.8±4.3	771±29	0.55±0.04	4.5±0.5	7.0±1.0
	86-164	8.50	0.348	12.0±0.9	66.7±3.0	410±16	0.37±0.03	3.7±0.4	5.5±0.9
4,832	164-242	8.06	0.307	12.2±1.1	74.6±3.6	390±17	0.31±0.02	3.4±0.4	4.7±0.7
	242-320	16.24	0.697	28.4±3.6	167±13	780±57	0.82±0.05	6.2±0.7	7.0±1.1
	1-8, 320-404*	9.89	0.427	14.6±2.1	106±9.0	495±38	0.47±0.03	2.5±0.3	4.1±0.7
	Mean*	11.19	0.492	17.7	101	588	0.51	4.5	6.1
	R ₁ †			0.994	0.948	0.897	0.995	0.872	0.752
	R ₂ ‡			0.918	0.838	0.987	0.943	0.810	0.828

Analyses were performed with methods described in ref. 12.

* Open cup during retrieval; mean values do not include data from this sample.

† Correlation coefficient (R_1) of radionuclide flux with bulk flux.

‡ Correlation coefficient (R_2) of radionuclide flux with organic carbon flux.

cells/d.p.m. dissolved per litre of surface sea water at time of apparent equilibrium with respect to partitioning between dissolved and particulate phases), the factor 4 is the dry weight:C ratio in cells, and P_n is the amount of new production (g C m⁻² yr⁻¹).

Equation (1) requires an estimate of new production. There have been numerous recent estimates of new production in different water columns, and there is still considerable discrepancy among estimates of various studies for a given region as well as significant spatial and temporal variability⁸. In applying equation (1), we use sediment-trap measurements of organic C flux from the euphotic zone for the water column in question (see below).

Laboratory studies using radiotracer methodology have shown a range of algal concentration factors among metals spanning six orders of magnitude, but a relatively narrow range ($\leq$ one order of magnitude) among diverse algal taxa for a given metal¹⁴. Concentration factors derived from laboratory studies are generally comparable with those from field measurements of metal concentrations in natural populations of phytoplankton^{15,16}, but do not always conform with their sediment K_d values¹⁷. Using equation (1), we have predicted the mean radionuclide:C ratio in sedimenting debris from a phytoplankton-based food chain (Table 2). ²³⁴Th shows the greatest signal and its predicted concentration of 1,740 d.p.m. g⁻¹ dry wt, or 7,000 d.p.m. g⁻¹ C, is comparable to measured values in sedimenting biodebris in the Pacific (1,600 d.p.m. g⁻¹ dry wt)¹⁸ and the Mediterranean (800–3,000 d.p.m. g⁻¹ dry wt)¹⁹. Similarly, the predicted value for ²¹⁰Pb (43.5 d.p.m. g⁻¹ dry wt, or 174 d.p.m. g⁻¹ C) is comparable to values from sedimenting debris in the Pacific (38 d.p.m. g⁻¹ dry wt)¹⁸ and Atlantic (229 d.p.m. g⁻¹ C)²⁰, and estimates of the average concentrations of the other radionuclides in planktonic debris (Table 2) are generally within a factor of two of measurements in zooplankton fecal pellets¹.

The degree to which predictions using equation (1) match sediment-trap measurements of radionuclide fluxes is a reflection of the relative importance of biogenic debris in transporting these radionuclides in a given water column. The agreement observed here between predictions (based on sediment-trap estimates of new production) and observations in different

waters underscores the general importance of biogenic debris as a vector for the vertical flux of these radionuclides in the open ocean (Table 3, Fig. 1), and suggests that artefacts such as grazing zooplankton in the traps have a relatively minor effect in these cases. The fluxes of ²²⁸Th, ²³⁴Th and ²¹⁰Pb recorded with deep traps match predictions, suggesting that there is relatively little net loss of these radionuclides from debris settling during descent, although organic carbon is lost^{8,21}. The ratios of predicted to observed fluxes of ²⁴¹Am and ^{239,240}Pu increase and decrease, respectively, with the depth of sediment trap, implying the progressive loss of ^{239,240}Pu from sinking particles and the uptake of ²⁴¹Am. An *in situ* source of ²⁴¹Am is provided through radioactive decay of the fallout radionuclide ²⁴¹Pu. Alternatively, the high ratio of predicted to observed Pu flux at Nares may be attributable in part to the fact that, unlike the Pacific, which received close-in fallout, the dissolved Pu in Atlantic surface water is $\geq$ 85% in the oxidized form¹²; the latter is thought to be less particle-reactive than reduced Pu (ref. 22).

In coastal waters, the importance of re-suspended sediment or horizontal transport processes may account for most of the vertical flux of radionuclides. For example, Table 3 shows that the measured flux of ²¹⁰Pb in slope waters off the north-east United States (2,750 m water column) is twice its predicted flux.

Table 2 Radionuclide concentrations estimated for plankton

Radionuclide	Experimentally determined geometric mean C_{dw} in phytoplankton	Representative surface seawater concentration, dissolved (d.p.m. l ⁻¹)	Predicted concentration in biogenic debris in surface waters (d.p.m. g ⁻¹ C)
²¹⁰ Pb	2.9×10^5 (ref. 26)	0.15 (ref. 28)	174
²¹⁰ Po	2.5×10^5 (ref. 27)	0.08 (ref. 28)	80
²²⁶ Ra	$< 6.2 \times 10^2$ (ref. 26)	0.075 (ref. 28)	< 0.2
²³⁴ Th	8.7×10^5 (ref. 26)	2 (ref. 10)	7×10^3
^{239,240} Pu	1.3×10^6 (ref. 15)	5×10^{-4} (ref. 29)	2.6
²⁴¹ Am	1.2×10^6 (ref. 15)	6×10^{-5} (ref. 29)	0.3

Table 3 Flux of radionuclides from surface waters

	Organic C flux from euphotic zone (g C m ⁻² yr ⁻¹)	Trap depth (m)	(d.p.m. m ⁻² yr ⁻¹)									
			²²⁸ Th*		²³⁴ Th† (× 10 ⁴)		²¹⁰ Pb‡ (× 10 ³)		^{239,240} Pu§		²⁴¹ Am	
			pred.	obs.	pred.	obs.	pred.	obs.	pred.	obs.	pred.	obs.
California current												
Vertex 1	77	50	—	—	54	53**	—	—	—	—	—	—
36°36' N, 123°48' W	(ref. 30)					(ref. 18)						
Vertex 1	154¶	80	—	—	—	—	—	—	18	18	18	4
35°48' N, 123°35' W	(ref. 31)								(ref. 31)		(ref. 31)	
MLML Pit Cruise-2	116	65	—	—	81	80	20	19	—	—	—	—
36°50' N, 123°00' W	(ref. 18)					(ref. 18)		(ref. 18)				
Vertex 2	15	120	—	—	10	68	—	—	—	—	—	—
18° N, 108° W	(ref. 8)					(ref. 5)						
Vertex 3	13	120	—	—	9	27	—	—	—	—	—	—
15°70' N, 107°50' W	(ref. 8)					(ref. 5)						
Monterey Bay	92#	250	—	—	—	—	21	22#	—	—	—	—
36°42' N, 122°13' W	(ref. 32)							(ref. 32)				
Central N. Pacific												
Vertex 4	14	150	—	—	10	24	—	—	—	—	—	—
28° N, 155° W	(ref. 8)					(ref. 5)						
Parflux P	1.82¶	378	—	—	—	—	—	—	0.71	0.50	0.22	0.33
15°21' N, 151° W	(ref. 33)								(ref. 33)		(ref. 33)	
Sargasso Sea	2.5	976	—	—	1.7	2.5	0.6	0.6	—	—	—	—
31°34' N, 55°03' W	(ref. 34)					(ref. 36)		(ref. 36)				
Tropical N. Atlantic	13	389	—	—	9	8	2.3	1.8	—	—	—	—
13°05' N, 54° W	(ref. 35)					(ref. 36)		(ref. 36)				
N.W. Atlantic slope	7	152	—	—	—	—	0.8	1.6	—	—	—	—
39°05' N, 70°55' W	(ref. 20)							(ref. 20)				
Nares Abyssal Plain	16	1,464	557	574	—	—	2.8	2.2	27	3.5	1.2	6.4
23°11' N, 63°58' W	(ref. 21)			(Table 1)				(Table 1)		(Table 1)		(Table 1)

* Dissolved surface concentration = 0.01 d.p.m. l⁻¹ (ref. 12).

† Dissolved surface concentration from Table 2.

‡ Dissolved surface concentration of 0.2 d.p.m. l⁻¹ (Monterey Bay³²; Sargasso Sea³⁶); 0.15 d.p.m. l⁻¹ (MLML Pit Cruise-2¹⁸; Tropical N. Atlantic³⁶; Nares¹²); 0.1 d.p.m. l⁻¹ (N.W. Atlantic slope³⁷).

§ Dissolved surface concentration of 9 × 10⁻⁵ d.p.m. l⁻¹ (Vertex 1³¹); 3 × 10⁻⁴ d.p.m. l⁻¹ (Parflux P³³); 3.3 × 10⁻⁴ d.p.m. l⁻¹ (Nares¹²).

|| Dissolved surface concentration of 1 × 10⁻⁴ d.p.m. l⁻¹ (Vertex 1³¹; Parflux P³³); 1.5 × 10⁻⁵ d.p.m. l⁻¹ (Nares¹²).

¶ Total mass flux.

During upwelling.

** Mean value.

In shallower waters over the continental shelf of the north-east United States, the measured ²¹⁰Pb flux²⁰ is considerably greater (by up to an order of magnitude) than predictions.

There are several empirical relationships that could provide quantitative estimates for some of the terms in equation (1) when direct data are lacking. Based on sediment-trap studies in the eastern Pacific, Pace *et al.*²³ developed an empirical relationship between new production and both total primary production and depth in the water column. They estimate the flux of particulate organic carbon (P'_n) as

$$P'_n = 3.523 z^{-0.734} P' \quad (2)$$

where z is depth in metres and P' = primary productivity, with P'_n and P' being expressed in mg C m⁻² d⁻¹. Thus, new production (P'_n) at 100 m accounts for an average of ~12% of total primary production in oligotrophic waters. The mean algal concentration factor of a given metal on a volume/volume basis (C_v) has been described as¹⁵

$$\log(C_v) = 8.547 e^{-17.086/x} \quad (3)$$

where x is the log of the solubility product of the metal hydroxide. To convert from a volume basis (vol) to a carbon basis (C), Strathmann²⁴ has shown that for diatoms, $\log(C) = 0.758 \log(\text{vol}) - 0.422$, whereas for other taxa, $\log(C) = 0.866$

$\log(\text{vol}) - 0.460$. Thus, for small cells ($\leq 200 \mu\text{m}^3$) the vol/C ratio in cells is ~7. At 100 m equation (1) can then be expressed as

$$\log(F) = \log(0.12P) + \log(D) + \log(7) + 8.547 e^{-17.086/x}$$

which simplifies to

$$\log(F) = \log(PD) + 8.547 e^{-17.086/x} - 0.0757 \quad (4)$$

where P = total primary productivity (g C m⁻² yr⁻¹). Thus, in the absence of other data, if P and D are known it should be possible to estimate the radionuclide flux (F) mediated by biogenic debris in a particular water column. High-quality measurements of the dissolved concentrations of many stable metals are now available²⁵ and equation (4) also provides a basis for estimating their fluxes from the euphotic zone.

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Hydroxy-aluminosilicate interlayers in montmorillonite: implications for acidic environments

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Hydroxy-aluminosilicate (HAS) ions are one of the major forms of soluble aluminium and the precursor of non-crystalline aluminosilicates in acidic ($pH < 5.5$) environments¹⁻⁶. Acidification in acid-vulnerable areas^{7,8} would thus promote HAS formation. Over four decades the study of interactions of soluble aluminium species with clay minerals has been focused on the deposition of hydroxy-aluminium in interlayer spaces of expandable layer silicates⁹⁻¹². Although the synthesis of 'hydroxy-silicoaluminium pillared smectites' as cracking catalysts has been recently attempted¹³, the nature of the dominant species adsorbed by smectites from the mixture of HAS, hydroxy-aluminium ions and silicic acid remains obscure. Here we provide evidence that HAS ions can be adsorbed in the interlayer spaces of montmorillonite, resulting in an expanded structure, and so the natural occurrence of HAS-interlayered layer silicates merits attention. Furthermore, the transport of HAS ions, their transformation to non-crystalline aluminosilicates and the levels of toxic solution aluminium species in acidic terrestrial and aquatic environments can thus be affected by naturally occurring expandable layer silicates such as montmorillonite.

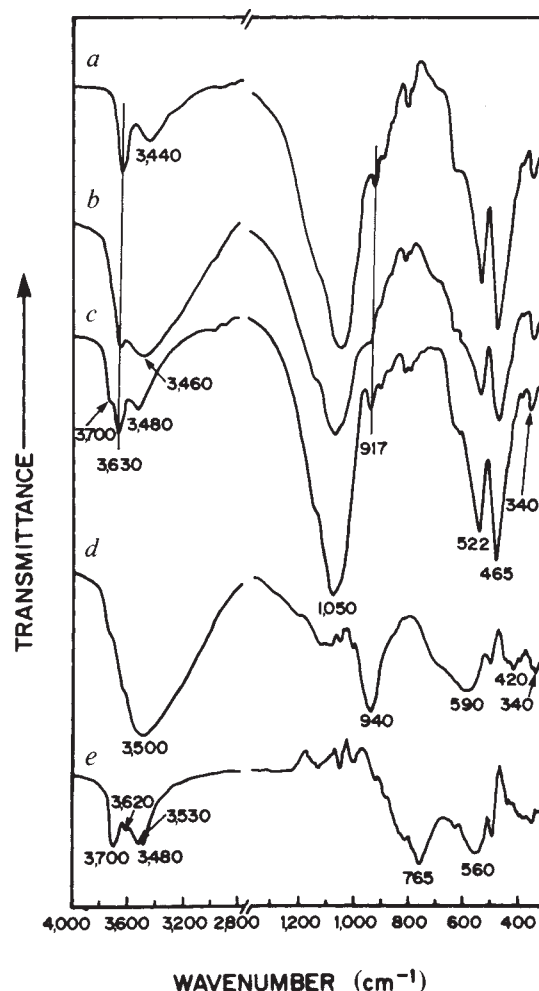
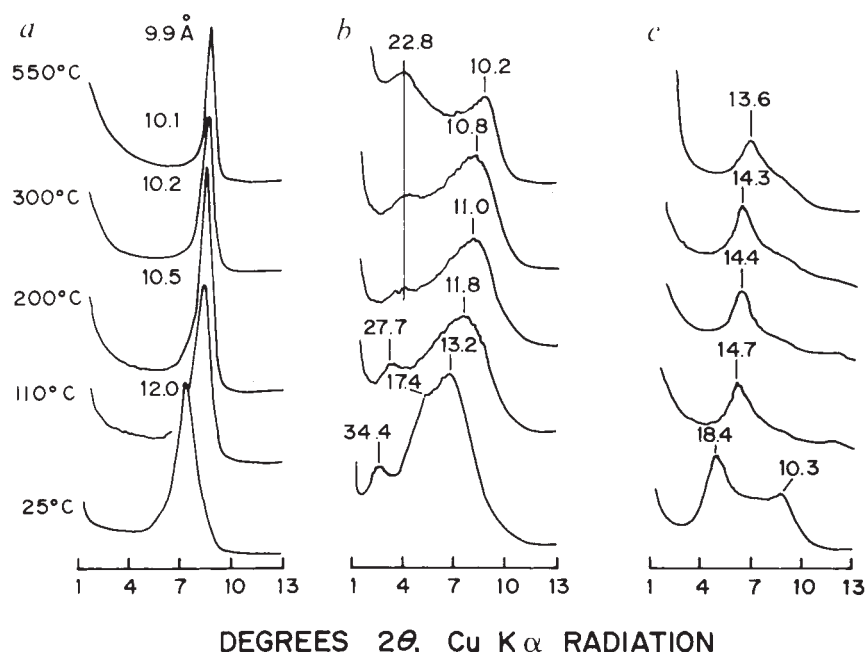


Fig. 1 Infrared spectra of *a*, the original montmorillonite, *b* montmorillonite treated with a solution of hydroxy-aluminosilicate ions, *c*, montmorillonite treated with a solution of hydroxy-Al ions; and differential infrared spectra of montmorillonite treated with solutions of *d*, hydroxy-aluminosilicate ions and *e*, hydroxy-Al ions. The clay complexes collected by filtration were saturated with K^+ ions before air-drying or heating. This procedure also removed the occluded solutions of hydroxy-aluminosilicate ions, hydroxy-Al or monosilicic acid. Infrared spectra were recorded on a Perkin-Elmer 983 spectrometer, using a KBr disc incorporating 1 mg of air-dried samples. Differential infrared spectra were obtained by placing the original clay in reference beam and treated clay in sample beam.

The solution of HAS ions used in this study had a Si/Al molar ratio of 0.56 and exhibited infrared absorption bands at 970, 568, 425 and 342 cm^{-1} , which resemble those reported by Wada and Wada¹ and Farmer *et al.*¹⁴. The absorption bands at 970 and 342 cm^{-1} are indicative of the formation of Si-O-Al bonds by reaction of hydroxy-aluminium ions with monosilicic acid.

In the present study, several approaches have been employed to establish the nature of the dominant species adsorbed by montmorillonite from the solution of HAS ions, which also contains unreacted hydroxy-aluminium ions and monosilicic acid. Table 1 indicates that substantial amounts of Si and Al were removed from the solution of HAS ions upon its interaction with montmorillonite. In contrast, the concentration of Si in the monosilicic acid-montmorillonite system with pH 6.69-6.85 remained virtually unchanged throughout the reaction period. Beckwith and Reeve¹⁵ observed significant dissolution of Si from bentonite below pH 7. Although the precise range of pH over which Si adsorption can occur may be obscured by the adsorp-

Fig. 2 X-ray diffraction patterns of *a*, the original montmorillonite, *b*, montmorillonite treated with a solution of hydroxy-aluminosilicate ions and *c*, montmorillonite treated with a solution of hydroxy-Al ions. K-saturated, parallel-oriented specimens were air-dried at 25 °C or heated at the specified temperatures for 2 h. The diffraction analysis was carried out on a Rigaku D/Max-RBX X-ray diffractometer.



tion-desorption equilibrium of monosilicic acid, it is evident that Wyoming bentonite has no net adsorption of monosilicic acid at $pH < 7$. It is therefore likely that in the present HAS ion-montmorillonite system at pH 4.01, Si was adsorbed in the form of HAS ions.

Further evidence for the adsorption of HAS ions by montmorillonite comes from infrared spectroscopy. The infrared absorption spectrum (Fig. 1a) of the original montmorillonite exhibits an Al-OH stretching vibration at $3,630\text{ cm}^{-1}$ and a bending vibration at 917 cm^{-1} , and an O-H stretching vibration of the adsorbed water at $3,440\text{ cm}^{-1}$ (ref. 16). Figure 1b and c shows the infrared spectra of the montmorillonite treated with the solution of HAS and hydroxy-aluminium ions, respectively. The infrared absorption bands from the adsorbed HAS ions are apparent at $3,500, 940, 590, 420$ and 340 cm^{-1} in the differential infrared spectrum (Fig. 1d). These infrared absorption characteristics resemble those of soil 'allophane-like' constituents^{17,18} and of an allophane-imogolite complex¹⁹. We consider that the adsorbed HAS has imogolite-like structures. In contrast, the differential infrared spectrum (Fig. 1e) of montmorillonite treated with a solution of hydroxy-aluminium shows absorption

bands at $3,700$ and $3,480\text{ cm}^{-1}$, which are identified as the O-H stretching vibrations of interlayer hydroxy-aluminium material²⁰. Weak absorption bands are present at $3,620$ and $3,530\text{ cm}^{-1}$, which are attributable to the presence of a small amount of gibbsite as a discrete phase²¹. The infrared spectrum of montmorillonite treated with the silicic acid solution (not shown) is virtually identical with that of the original montmorillonite (Fig. 1a). This is consistent with the chemical data presented in Table 1, which show no adsorption of silicic acid by montmorillonite.

The montmorillonite treated with the solution of HAS ions and that treated with hydroxy-aluminium ions have significantly different X-ray diffractograms. Whereas the original montmorillonite showed a 12.0-Å basal d (layer) spacing upon saturation with K^+ ions and air-drying at 25 °C (Fig. 2a), the montmorillonite treated with the solution of HAS ions gave X-ray diffraction peaks at $34.4, 17.4$ (shoulder) and 13.2 Å upon K-saturation and air-drying at 25 °C , and at 27.7 and 11.8 Å after heating at 110 °C for 2 h (Fig. 2b). The 27.7-Å peak is attributable to a regular interstratification (alternation) of the HAS-interlayered component, with a basal d spacing of 17.2 Å , and a non-

Table 1 Adsorption of Si and Al by montmorillonite from HAS, hydroxy-Al and monosilicic acid solutions

Solution	pH		Solution Si (mmol l ⁻¹)		Solution Al (mmol l ⁻¹)		Si adsorbed (mol kg ⁻¹)	Al adsorbed (mol kg ⁻¹)
	Initial	Final	Initial	Final	Initial	Final		
HAS	4.01	4.01	1.43	0.83	2.56	1.29	1.96	4.14
Hydroxy-Al	4.40	4.21		0.06	2.58	1.44		3.72
Silicic acid	6.69	6.85	1.43	1.47				

The solution of HAS ions was prepared from $0.1\text{ M AlCl}_3 \cdot 6\text{H}_2\text{O}$ solution and $1.85 \times 10^{-3}\text{ M}$ monosilicic acid, which was obtained by passing sodium metasilicate solution through a H^+ -form Dowex 50W-X8 ion-exchange resin column²⁶. The mixed solution, with a Si/Al molar ratio of 0.56, was titrated with 0.1 M NaOH under continuous stirring at the rate of $\sim 0.5\text{ ml min}^{-1}$ to an OH/Al molar ratio of 2.0. The resulting solution had a pH of 4.36, which decreased to 4.00 after ageing for 10 days. The preparation of the solution of hydroxy-Al was identical except that monosilicic acid was omitted. An aliquot of 500 ml of the solution of HAS, hydroxy-Al or monosilicic acid was mixed with 50-ml suspension of $<2\text{-}\mu\text{m}$ Na-saturated montmorillonite (Crook, Wyoming, USA) to obtain a 306-mg l^{-1} clay suspension. The resultant suspensions were agitated for the first 72 h and then allowed to stand at 25 °C to complete a total reaction period of 6 weeks. At the end of this period, the solutions and solid phases were separated by filtration through a Sartorius cellulose nitrate membrane filter of $0.01\text{-}\mu\text{m}$ pore size. The pH of the three solution-montmorillonite systems was measured at the beginning and the end of the reaction period. Si and Al concentrations in solution were determined colorimetrically^{27,28}, after decomposing HAS ions by heating a 2-ml sample in 20 ml 0.5 M NaOH at 90 °C for 15 min. The amounts of Si and Al adsorbed were calculated by taking the difference between the initial and final Si and/or Al concentrations.

interlayered component, with a 10.5-Å basal d spacing, which is the same as that of the original montmorillonite heated to the same temperature (Fig. 2a). Taking the thickness of a montmorillonite layer as 9.6 Å, the thickness of the HAS interlayer in the 17.2-Å interlayered component is then 7.6 Å, which can be assigned as the thickness of a monolayer of HAS polymer cations. The HAS ions are likely to have a structure similar to that of imogolite on the molecular scale¹⁴, that is, an orthosilicate group attached to the fragment of a gibbsite-like sheet of aluminium hydroxide²². The 11.8-Å peak (Fig. 2b) could arise from the interlayer adsorption of partially hydrolysed Al and/or small-sized HAS ions formed from the reaction of monosilicic acid with partially hydrolysed Al. The shift of 27.7- and 11.8-Å peaks to lower d spacings upon heating to >110 °C is indicative of the partial dehydroxylation of the interlayer polymers. The reaction of montmorillonite with hydroxy-aluminium ions on heating to 110–300 °C resulted in a clay complex with basal d spacings of 14.3–14.7 Å (Fig. 2c), which indicates interlayering of aluminium hydroxide^{11,12}. The monosilicic acid-treated montmorillonite exhibited an X-ray diffraction pattern (not shown) essentially identical to that of the original montmorillonite (Fig. 2a), implying that interlayer adsorption of silicic acid did not occur (Table 1).

Our data reveal that Al and Si are adsorbed as HAS ions in the interlayer spaces of montmorillonite. The large basal spacings of the HAS–montmorillonite complex provide evidence of an expanded structure, which could have potential applications in catalysis. Moreover, our results indicate that expandable layer silicates such as montmorillonite, which extensively occur in soils and sediments or as suspended particulates in natural waters, can affect the transport and transformation of HAS ions and thus can influence the levels of solution aluminium species and related ecological and toxicological problems²³ in acid-vulnerable terrestrial and aquatic environments. As HAS ions can form under pH conditions^{1,2} that are most conducive to hydroxy-aluminium interlayering¹¹, the incorporation of Si along with Al in interlayers of expandable layer silicates in nature is inevitable. Hence, our data also provide a partial explanation for the presence of substantial amounts of Si in the materials extracted from natural hydroxy-aluminium-interlayered minerals^{24,25}. The natural occurrence of HAS-interlayered layer silicates thus deserves attention, and in particular the influence of adsorption of HAS by montmorillonite on allophane and/or imogolite formation merits future research.

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Evidence from shear-wave splitting for the restriction of seismic anisotropy to the upper crust

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The existence of anisotropy in the propagation speed of seismic waves through the Earth's crust has been attributed to the non-random alignment of microcracks in the crust¹. For sparsely distributed, parallel vertical cracks, perturbation theory predicts the splitting of a shear wave into two orthogonally polarized waves^{2–4}. Here we present extremely clear evidence for shear-wave splitting in three-component seismograms from crustal and sub-crustal microearthquakes recorded in the Shikoku district of Japan. Quantitative analysis of travel-time differences between split shear waves leads to the conclusion that the observed anisotropy is limited to the upper crust, shallower than 10–15 km. This model is consistent with recent results from reflection seismology⁵, and implies that microcrack density or orientation changes from the upper to the lower crust.

Shear-wave splitting can be observed either as a parallel alignment of particle motions of the leading shear waves coming from various azimuths and incident angles⁶, or as an abrupt change in polarization on the arrival of slower split shear waves². Many researchers have reported shear-wave splitting^{7–9}, mainly on the basis of the parallelism of the leading shear waves. The arrivals of slower split shear waves tend to be obscured by waveform contamination, arising from the presence of strong heterogeneities in the upper part of the crust. Consequently, travel-time differences between orthogonally polarized shear waves have been difficult to estimate. It is important to do so, however, because travel-time differences are quite informative as to the degree of crustal anisotropy. Once the degree of anisotropy is reliably estimated, it can be translated into *in situ* crack parameters, such as density and aspect ratio, assuming that the cause of the anisotropy is a non-random alignment of microcracks^{3,4,10}. In the data presented here, not only the parallelism of the leading shear waves but also the arrival of slower split shear waves can be clearly seen in many seismograms, which enables us to retrieve travel-time differences and to estimate the degree of crustal anisotropy.

We use data from the seismic network of the Kochi Earthquake Observatory which covers the central and south-east parts of the Shikoku district (Fig. 1). Three-component velocity seismograms were recorded at five telemetered stations (indicated as stars in Fig. 1) and the data were digitalized. Figure 2 indicates typical examples of the waveforms obtained at AKR together with diagrams of particle motions in the horizontal plane over specified time intervals; the latter have been rotated into transverse and radial components. Most of the shear waves at AKR have simple impulsive waveforms. This may be attributed to simple structure in the crust beneath AKR, which is located at a site that is free from large topographic irregularities. The simplicity of these waveforms and the nature of the particle motions are consistent with the features of split shear waves predicted by theories of seismic anisotropy². The arrival of the slower split shear-wave, which is characterized by an abrupt change in polarization direction, is readily apparent on the particle-motion diagrams. The east–west component of the shear wave arrives 0.1–0.3 s earlier than the north–south component (S.K. and M.A., manuscript in preparation). Instrumental and sampling delays between the two components are

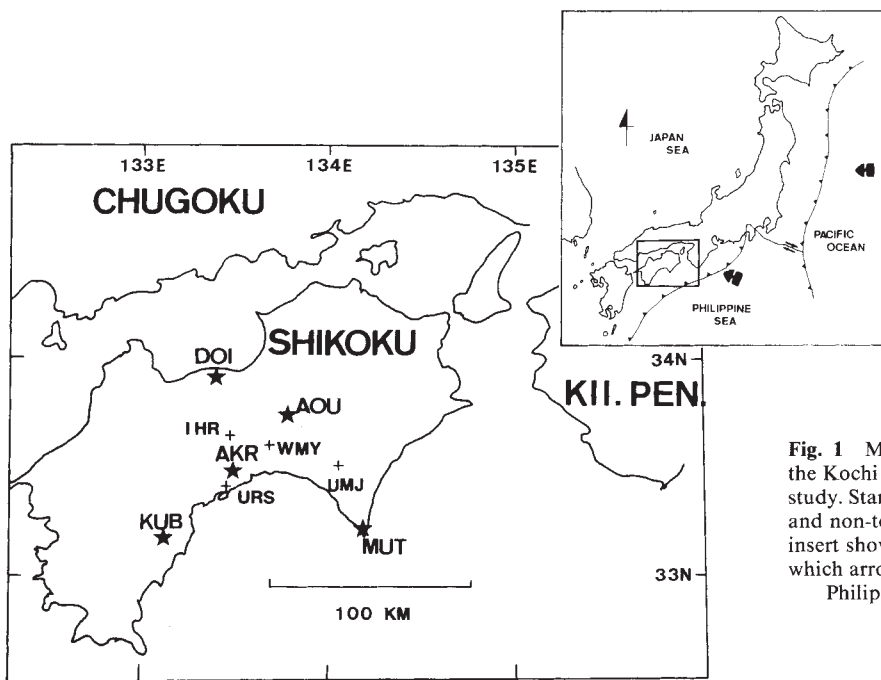


Fig. 1 Map showing the seismic stations of the Koshi Earthquake Observatory used in this study. Stars and plus signs indicate telemetered and non-telemetered stations respectively. The insert shows the location of the study area, on which arrows indicate the slip directions of the Philippine Sea plate and Pacific plate.

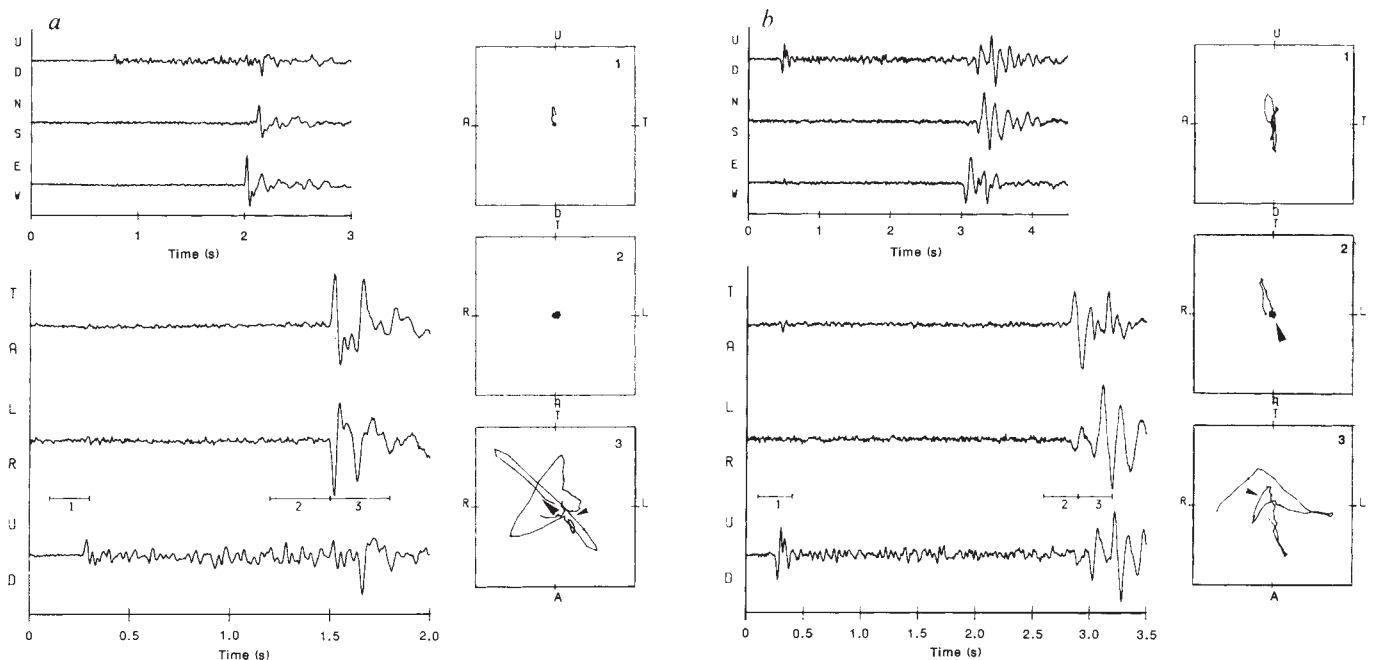


Fig. 2 Examples of three-component seismograms ((U) up-(D) down, (N) north-(S) south and (E) east-(W) west), rotated seismograms ((T) toward-(A) away from epicentre and (R) right-(L) left viewing station from epicentre and (U) up-(D) down), with particle motion diagrams for the interval indicated in rotated seismograms. Large and small arrowheads depict the arrival of the faster and slower split shear waves, respectively. Azimuth, incident angle at the surface, focal depth and magnitude of each event are indicated.

1 ms at most, so that the difference in the arrival times is not an instrumental artefact. As most of the leading east-west polarized S-phases have transverse and radial components with very small vertical amplitudes, there is no possibility that they are S-to-P-converted waves generated at internal discontinuities.

Such unequivocal examples of shear-wave splitting have been reported for shear waves reflected at the core-mantle boundary (ScS phases) from deep earthquakes¹¹ and for S-waves from upper-mantle earthquakes with depths of 200–300 km (ref. 12), and are considered as evidence for upper-mantle anisotropy resulting from a preferred orientation of minerals such as olivine or from melt-filled crack alignment^{11,12}. By using crustal (<25 km) and sub-crustal (focal depths 30–45 km) earthquakes, this study presents unequivocal evidence for the presence of shear-wave splitting, for which crustal anisotropy could be

responsible. An advantage of studying crustal anisotropy rather than upper-mantle anisotropy is that much more waveform data is accessible for the former.

At the other stations in the central part of the Shikoku district (AOU, DOI, URS and UMJ in Fig. 1) the leading shear waves are polarized uniformly or nearly uniformly in the east-west direction, although the alignments are less clear than at AKR. At these stations, however, reverberations are prominent after the arrival of the first shear wave, obscuring the arrival of the slower split shear waves. These reverberations may be due to heterogeneities in the crustal media at stations other than AKR; that is to say, only if the crust is sufficiently simple can the arrival of the slower orthogonally polarized shear waves be reliably resolved.

The shallow part of the upper crust in the central part of the

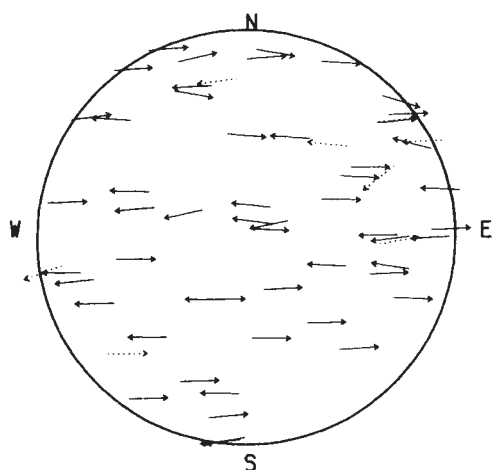


Figure 3 Equal-area projection of the leading shear-wave particle-motions at AKR. The particle motion directions are shown with arrows. Dashed arrows indicate less reliable data.

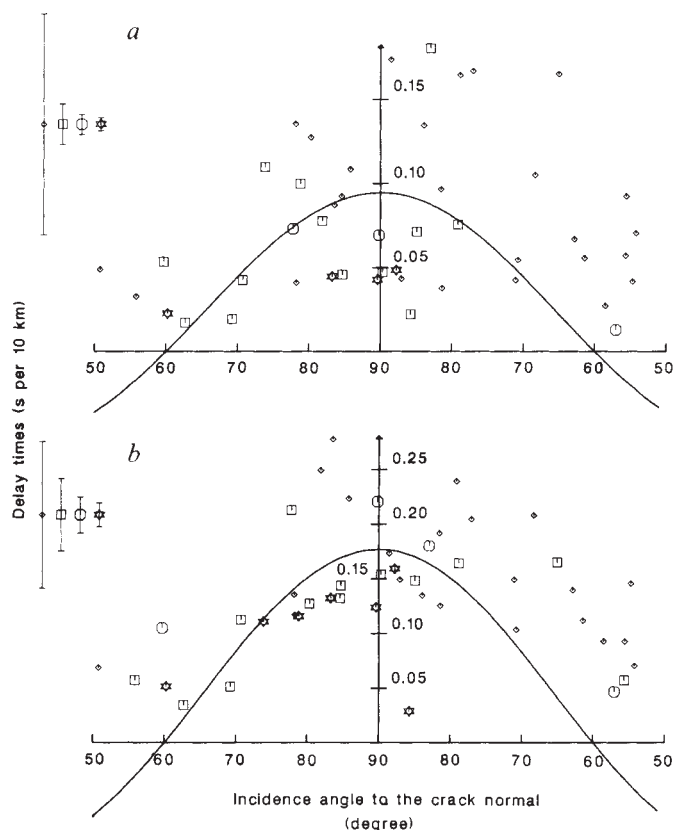


Fig. 4 Normalized travel-time differences versus angle between seismic rays and the normal of vertical cracks striking east-west. Solid lines indicate theoretical travel-time differences normalized to 10-km propagation through a homogeneous medium containing thin (zero aspect ratio) liquid-filled cracks. Respective error bars are shown for each symbol to the left of the figure. The thicknesses of the anisotropic layer are *a*, 50 km, and *b*, 10 km.

Shikoku island is considered to be subject to east-west compressive tectonic stress, mainly through westward subduction of the Pacific plate at the Japan Trench¹³. Focal mechanism analysis indicates that crustal earthquakes (shallower than 20 km) in this region have P-axes striking approximately east-west¹⁴. The orientation of the leading shear-wave polarization is nearly coincident with the horizontal maximum compression. Such correspondence between the tectonic stress and the shear-wave splitting for crustal earthquakes has been found recently at many

places in the world^{7,8}. One of the proposed causes of shear-wave splitting is the existence of parallel vertical microcracks^{1,3}, aligned perpendicular to the minimum compression in the presence of tectonic stress¹⁵. Our results support this hypothesis, but we cannot rule out the possibility that the shear-wave splitting is instead a manifestation of crustal anisotropy from mineral alignment¹⁶.

Travel-time differences between the leading and slower split shear waves are retrieved on the seismograms at AKR by two methods: (1) visual inspection of the arrivals of the leading and slower shear waves on the particle-motion diagrams in the horizontal plane, and (2) calculating arrival-time differences between two horizontal components (north-south and east-west) by a waveform correlation analysis. When there is a large discrepancy between the results of the two methods, the data are disregarded. For simplicity, seismic anisotropy is assumed to be uniform in a layer extending from the surface down to a certain depth, which is varied to estimate the thickness of this anisotropic layer. To calculate the degree of anisotropy, the travel-time differences are normalized to ray path lengths in the estimated thickness of the anisotropic layer between the source and the AKR station. The normalized travel-time differences (delay times) exhibit a large degree of scatter when the anisotropic layer extends to >20 km depth (Fig. 4*a* shows an example for a 50-km-thick layer), whereas for anisotropy depths of 10–15 km the travel-time differences are more narrowly distributed (Fig. 4*b*). The normalized delay times are fitted more convincingly by a model that incorporates parallel alignment of vertical cracks striking in the east-west direction than by the model with a thicker anisotropic layer. Thus, we propose that the assumption of uniform anisotropy throughout the crust is inconsistent with the results from the shear-wave analysis, and that anisotropy is limited instead to the upper 10–15 km of the crust^{9,17}. If vertical microcracks are the source of anisotropy, the density of vertical microcracks in the lower crust would be then much smaller than that in the upper crust. Unfortunately our data set cannot resolve the exact range in depth of the anisotropy, because most events used are deeper than 10 km. We cannot reject the possibility that a strong anisotropy is concentrated within a thin surface layer.

The suggestion that anisotropy is concentrated in the upper 10 km seems compatible with the observations, from reflection seismology¹⁸, of a transparent upper crust without prominent horizontal reflectors and a reflective lower crust with many strong reflectors. Here it is suggested that anisotropy is generated by an alignment of cracks significantly smaller than the typical wavelength of a shear wave (several hundred metres), and we speculate that some of these may be nevertheless large enough to behave as a reflector. Thus a horizontally laminated lower crust, possibly with horizontal microcracks, may have little anisotropic effect on nearly vertically incident shear waves but may nevertheless contain strong reflectors, whereas on the other hand, an upper crust with non-horizontally aligned cracks could have effective anisotropy whilst being non-reflective.

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Egg mimicry by cuckoos *Cuculus canorus* in relation to discrimination by hosts

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Although the cuckoo *Cuculus canorus* parasitizes a variety of hosts, individual females are thought to favour just one species and they lay eggs coloured to match, more or less accurately, those of their particular host¹⁻³. The mechanisms underlying this division of females into strains, or gentes, specializing on different hosts are unknown. From a study of museum collections we show that the eggs of British cuckoo gentes are statistically different and, using model eggs, we show by experiment that host discrimination against badly matching eggs is a selective force in gens maintenance and that cuckoos lay a better mimetic egg where the host species is apparently more discriminating.

In Britain, cuckoos exploit five main hosts: meadow pipits *Anthus pratensis* in moorland and heathland, reed warblers *Acrocephalus scirpaceus* in marshland and dunnocks *Prunella modularis* in woodland and farmland, with robins *Erithacus rubecula* and pied wagtails *Motacilla alba* being important subsidiary hosts⁴. With the exception of the dunnock, there is a generalized match between parasite and host egg (Fig. 1). To test statistically the apparent difference between cuckoo eggs laid in different British host nests, we examined collections at the British Museum (Tring, Hertfordshire) and the Oxford University Museum. The eggs of the five different gentes did not differ significantly in size (mean length $\times$ breadth² in cm = $6.36 \pm$ s.d. 0.624, $n = 189$, $F_{4,184} = 1.006$, NS), or in heaviness of spotting measured subjectively on an ordinal scale from 0 (diffuse smudging, no heavy spots) to 3 (numerous, heavy, distinct spots; $\chi^2 = 0.25$, 4 d.f., NS). In all gentes the most common ground colour was grey-green (Table 1), but primarily because of an excess of slightly yellower eggs in reed warbler nests (where the host eggs are somewhat yellower than in the other hosts), the

chi-square value is significant. The eggs of the cuckoo gentes differed significantly in overall darkness, and the rank order corresponded closely ($r_s = 0.92$, $n = 5$, $P < 0.05$) with the ranking of the darkness of their respective host eggs (Fig. 2). Interestingly, cuckoo eggs in meadow pipit nests in northern Britain (Cheshire, Lancashire and Yorkshire, northwards), where the pipit is the main host, were darker ($164.0 \pm$ s.e. 4.5, $n = 40$) and therefore a closer match to the pipits' eggs than in southern England (185.4 ± 8.4 , $n = 24$; $t = 2.46$, 62 d.f., $P < 0.02$), where the pipit is a lesser and perhaps more often an accidental host.

The data in Fig. 2 refer to cuckoo eggs collected, not to cuckoo eggs laid. If the hosts had ejected a proportion of cuckoo eggs before collection, a proportion biased in favour of unlike eggs (see below), the observed correlation between host and cuckoo egg could result. Three arguments counteract this criticism. In our detailed studies of reed warbler/cuckoo interactions, less than 10 per cent of real cuckoo eggs were rejected⁵, probably too few to establish the significant differences between gentes seen in Fig. 2. Next, within the collected clutches of the three species known to treat model eggs of different darkness differentially (see Table 2), there was no correlation between cuckoo and host egg darkness (reed warbler $r = 0.09$, $n = 44$; meadow pipit $r = 0.11$, $n = 42$; great reed warbler $r = 0.24$, $n = 12$, all NS), which might be anticipated if hosts were discriminating very accurately. Finally, detailed studies of individual female cuckoos^{2,3,6} have shown that they lay most of their eggs in the nests of one host species and do not scatter eggs among several hosts, which only then incubate the cuckoo egg if it matches their own.

We have assessed how well cuckoo eggs matched host eggs. Dunnock-cuckoo eggs were excluded from the assessment as they do not mimic those of the host (Fig. 1). Robin-cuckoo eggs were also excluded. Their mean darkness is similar to pied wagtail-cuckoo eggs (Fig. 2), but the variance in darkness is significantly greater ($F_{47,102} = 1.77$, $P < 0.05$). Consequently it is not certain that the robin gens lays distinct eggs. Hungarian great reed warbler-cuckoos, however, display more accurate mimicry than any observed in Britain⁷ (Fig. 1) and were used in the assessment, which proceeded as follows. We asked 10 colleagues to match 40 cuckoo eggs (10 each from pied wagtail,

Table 1 The ground colour of cuckoo eggs belonging to five British gentes

Number of eggs of ground colour corresponding with	Gens					Total
	Robin	Pied wagtail	Dunnock	Reed warbler	Meadow pipit	
Bluer plates 20-27	17	38	23	16	25	119
Modal green plate 28	21	55	50	22	48	196
Yellower plates 29-35	11	11	16	25	29	92

$\chi^2 = 25.3$, 8 d.f., $P < 0.005$. Plate numbers refer to colourplates of the Methuen Handbook of Colour¹⁷. The most common ground colour ($n = 179$), the greenish-white, scored as 28A2 on plate 28, has a Munsell reference 5GY 9/1.

Table 2 The proportion of nests at which various types of model cuckoo eggs (Fig. 1) were rejected by hosts

Host	Proportion of nests where model cuckoo egg rejected					Significance of difference	Numbers of rejections by		
	Pied wagtail type	Redstart type	Reed warbler type	Meadow pipit type	Great reed warbler type		Ejection	Desertion	Other
Robin	2/7	1/7	—	2/11	—	NS	2	3	—
Pied wagtail	7/14*	10/13	5/7	12/18	—	NS	12	21	1§
Dunnock	0/7	0/5*	0/4	1/6	—	NS	—	1	—
Reed warbler	13/16	17/28	0/19*	4/11	—	$P < 0.001^\dagger$	16	7	11
Meadow pipit	9/25	15/18	4/15	6/27*	—	$P < 0.001^\dagger$	5	29	—
Great reed warbler	—	5/5	—	1/2	1/5*	$P = 0.024^\ddagger$	6	1	—

Experiments were done in Cambridgeshire (reed warbler, robin, dunnock), mostly on Derbyshire moorland (meadow pipit), all over Britain (pied wagtail) and in Burgenland, eastern Austria (great reed warbler). NS, not significant.

* Model cuckoo eggs mimicking the host eggs. $^\dagger \chi^2$ test, 3 d.f. ‡ Fisher-exact test, excluding meadow pipit treatment.

§ Built new nest on top of parasitized clutch ('built over'). ^{||} 2 built over, at 9 nests birds were seen pecking at model⁵.



Fig. 1 Host eggs, cuckoo eggs and model eggs. The left-hand column shows eggs of passerines parasitized by the cuckoo. From the top downwards these are the five principal British hosts⁴: robin, pied wagtail, dunnoek, reed warbler, meadow pipit, and also the great reed warbler, which is a central European host⁷. The central column is a typical example of a cuckoo egg laid in the corresponding host nest. The right-hand column is an example of a model egg painted to mimic the corresponding cuckoo egg. As robin and pied wagtail-cuckoo eggs are not clearly distinct (Fig. 2), we painted just one type of egg, a pied wagtail model, shown here bridging the robin row (1) and the pied wagtail row (2). In the case of the dunnoek (row 3), where there is no colour mimicry between cuckoo and host egg, our pale blue model eggs match those laid by cuckoos parasitizing redstarts *Phoenicurus phoenicurus* (which lay immaculate eggs) in Finland⁷. The model eggs, of the exact size and weight laid by a cuckoo, were made of gel coat resin and painted with acrylic paints⁵.

reed warbler, meadow pipit and great reed warbler nests) to one of four displayed host eggs (one per host; four different host eggs used for each colleague). If matching were random, 25/100 correct choices would be made for each cuckoo gens. In fact the scores for great reed warbler, meadow pipit, pied wagtail and reed warbler were 69, 57, 41 and 30 respectively. Except for the reed warbler, these scores are significantly different from random ($P < 0.01$), and they provide an objective ranking of mimicry.

Mimicry could be maintained if hosts rejected unlike eggs⁸, for which there is some evidence^{1,9,10}. To test this experimentally, we made model cuckoo eggs to represent the various gentes (Fig. 1) and placed them in host nests one afternoon, mainly during the laying period or early incubation (there was no

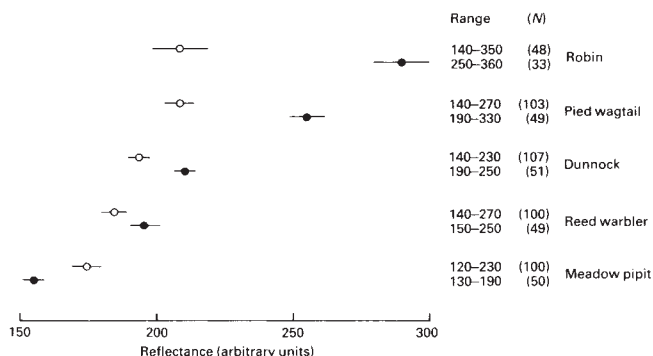


Fig. 2 The reflectance of the eggs (●; bars indicate 95% confidence limits) of five principal hosts of cuckoo in Britain and the reflectance of cuckoo eggs (○) found in their nests. Sample sizes and ranges are given for each species. To measure reflectance (our term), as an indicator of overall darkness, we placed the egg in a standardized white light on a white card of luminance 310 cd m^{-2} . Light reflecting from the egg was measured by a Gossen Mastersix lightmeter with a Profiflex attachment, whose aperture was 12 mm from the upper surface of the egg. Higher values indicate more light reflected and thus a paler egg. Meadow pipit-cuckoo eggs (mean reflectance $174 \pm \text{s.e. } 2.7$) were significantly darker ($t = 3.09$, 198 d.f., $P < 0.01$) than reed warbler-cuckoo eggs (185 ± 2.3), which were significantly darker ($t = 2.54$, 205 d.f., $P < 0.02$) than dunnoek-cuckoo eggs (193 ± 2.0), which in turn were significantly darker than pied wagtail-cuckoo eggs (208 ± 2.6 ; $t = 4.60$, 208 d.f., $P < 0.001$) and robin-cuckoo eggs (208 ± 5.0 ; $t = 3.30$, 153 d.f., $P < 0.01$). Robin- and pied wagtail-cuckoo eggs did not differ significantly ($t = 0.2$, 149 d.f., NS). Entirely because of higher robin-cuckoo variance, there was significant difference in darkness variance between cuckoo eggs laid in the five hosts ($F_{\text{max}, 5, 47} = 2.47$, $P < 0.05$). Although cuckoo eggs and the corresponding host eggs match quite well in reflectance, the degree of matching and the cross-over from species where the host egg is darker than the cuckoo's (for example, meadow pipit) to those where it is paler (for example, pied wagtail) cannot be determined exactly from the figure because, compared with host eggs, cuckoo eggs are noticeably more glossy and have longer radii of curvature which may affect the measured reflectance.

difference in host response at these different times¹¹). A model was scored as accepted if the eggs had not been deserted or if the model had not been ejected within 3 days of experimental parasitism or clutch completion, whichever was later (92% rejections occurred by this criterion¹¹). Most rejections were by ejection or desertion (Table 2). Both reed warblers and meadow pipits were significantly less likely to desert nests containing like models (representing their respective cuckoo gens) than unlike models ($P < 0.05$ in each case), suggesting that desertion was largely a rejection response to the models¹¹. Three hosts, great reed warbler, meadow pipit and reed warbler, showed significant differences in rejection of the different model types and all three showed greatest acceptance of the mimetic models, those representing their own cuckoo gens. Pied wagtails did not show significant difference in response to the model types, although again the mimetic model was the type most accepted. Of the three well mimicked British hosts, however, only reed warblers showed significantly greater acceptance of the mimetic model in comparison with each of the three non-mimetic types. The robin and, more so, the dunnoek accepted most models. Dunnocks also accepted black models, white models and whole clutches of models unlike their own eggs¹¹.

Thus, host species for which the cuckoo lays a mimetic egg (Fig. 1) tended to reject unlike eggs; the dunnoek, not mimicked, showed little, if any, rejection. Among rejecting species, the rank order of frequency of rejection of redstart-type models was great reed warbler, meadow pipit, pied wagtail, reed warbler, which correlates exactly ($r_s = 1$, $n = 4$, $P = 0.05$) with the quality of cuckoo egg mimicry as assessed by colleagues (above).

The lack of discrimination shown by the dunnoek explains why this cuckoo gens has not evolved a mimetic egg, but it raises two questions. First, why does the dunnoek not discriminate against cuckoo eggs, given the reproductive costs of not doing so? We cannot resolve three possible answers. Either the dunnoek may lack the genetic variation from which egg discrimination might evolve¹² or, for some reason, discrimination by dunnoeks is peculiarly costly or the dunnoek has become a host so recently that discrimination, even if it has appeared, has not had time to spread through the population at large. In fact we know the dunnoek has been a host for at least 600 years; in Chaucer's poem¹³ 'The Parlement of Foules' (line 612), the cuckoo is chastised as "thow morderer of the heysugge on the braunche that broghte thee forth", 'heysugge' being Old English for hedge sparrow (dunnoek). With the present 2% parasitism rate⁴, however, it could take a discrimination gene several thousand generations to spread through the population¹⁴, so the time-lag hypothesis cannot be excluded.

Second, given that dunnoeks do not reject unlike eggs, why is there a close correspondence between the darkness of dunnoek-cuckoo and dunnoek eggs, and why is the variance in that darkness no greater than that of other gentes (Fig. 2)? It is possible that the correspondence is coincidental. If cuckoos specializing on dunnoeks occasionally parasitized other more discriminating hosts, selection could favour whatever egg type was most likely to be accepted by other hosts. If the other hosts were reed warblers and wagtails living in the same lowland regions as dunnoeks, then an egg intermediate between that of the reed warbler and wagtail-cuckoo gentes could be selected. By this explanation, the fact that the cuckoo egg's darkness corresponds with the dunnoek's is pure chance. Alternatively, the matching in darkness could be selected for by predation if predators more readily noticed nests containing a discordant egg¹⁵. Avian predators, with good colour vision, would presumably select for colour mimicry by cuckoos. But mammalian predators, with no colour vision, could in theory select for shade-matching if a cuckoo egg of different darkness to the dunnoek's own eggs made the clutch more conspicuous. Recent experiments with other species, however, provide no support for the idea that predation selects for mimicry by brood parasites^{5,16}.

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Pancreatic amylin and calcitonin gene-related peptide cause resistance to insulin in skeletal muscle *in vitro*

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Insulin resistance occurs in a variety of conditions, including diabetes, obesity and essential hypertension¹, but its underlying molecular mechanisms are unclear. In type 2 (non-insulin-dependent) diabetes mellitus, it is insulin-resistance in skeletal muscle, the chief site of insulin-mediated glucose disposal in humans², that predominantly accounts for the low rates of glucose clearance from the blood, and hence for impaired glucose tolerance³. Human type 2 diabetes is characterized by a decrease in non-oxidative glucose storage (muscle glycogen synthesis)^{4,5}, and by the deposition of amyloid in the islets of Langerhans^{6,7}. Amylin is a 37-amino-acid peptide which is a major component of islet amyloid and has structural similarity to human calcitonin gene-related peptide-2 (CGRP-2; ref. 8). CGRP is a neuropeptide which may be involved in motor activity in skeletal muscle^{9,10}. We now report that human pancreatic amylin and rat CGRP-1 are potent inhibitors of both basal and insulin-stimulated rates of glycogen synthesis in stripped rat soleus muscle *in vitro*. These results may provide a basis for a new understanding of the molecular mechanisms that cause insulin resistance in skeletal muscle.

Glycogen metabolism in skeletal muscle *in vivo* is thought to be regulated by the hormones adrenaline and insulin¹¹. We tested the effects of CGRP-1 and an adrenaline analogue (the pure β -adrenoceptor agonist isoprenaline) on insulin-stimulated glucose metabolism in an incubated stripped soleus muscle preparation of the rat (Fig. 1a, b). CGRP-1 markedly inhibited the insulin-stimulated rates of [14 C]glucose incorporation into glycogen, but only stimulated the rate of glycolysis at a maximal concentration of 10^{-6} M. The concentration of effector required for half-maximal response (EC_{50} value) of CGRP-1 for glycogen synthesis in this system was 1×10^{-9} M (Fig. 1b). In marked contrast, isoprenaline significantly stimulated the rates of glycolysis at sub-micromolar concentrations. Isoprenaline also caused inhibition of insulin-stimulated rates of glycogen synthesis and stimulation of glycolysis with EC_{50} values of 4×10^{-9} M and 5×10^{-9} M, respectively. Therefore, it may be that a cyclic AMP-dependent mechanism does not mediate CGRP-effects (Fig. 1), as isoprenaline is known to elevate cAMP at concentrations (10^{-8} M) that both increase the rate of glycogenolysis and inhibit glycogen synthesis¹². In contrast, equivalent concentrations of CGRP-1 specifically inhibit glycogen synthesis (Fig. 1), without altering the rate of glycolysis.

In the absence of CGRP-1, a maximal concentration of insulin ($>1,000 \mu\text{U ml}^{-1}$) stimulated the rate of glycolysis by ~50% and the rate of glycogen synthesis more than twofold (Table 1). CGRP-1 ($1 \mu\text{M}$) significantly decreased glycogen synthesis at all concentrations of insulin, but had no effect on the rate of conversion of [14 C]glucose to [14 C]lactate (indicating the rate of glucose transport into muscle fibres¹³; results not shown). Also, CGRP-1 (10^{-8} M) had no effect on the insulin-stimulated rates of transport of a glucose analogue, 3-O-methyl-D-glucose (data not shown). Lower concentrations of CGRP-1 had no effect on the rates of glycolysis; however, CGRP (10^{-9} M) significantly decreased the rates of glycogen synthesis at all concentrations of insulin (Table 1). At 100 pM, CGRP-1 significantly inhibited glycogen synthesis only when $100 \mu\text{U ml}^{-1}$ of insulin was present.

CGRP-1 therefore specifically inhibits glycogen synthesis at concentrations similar to the K_d values (range 1.8-18 nM) reported from CGRP binding studies carried out with a variety of

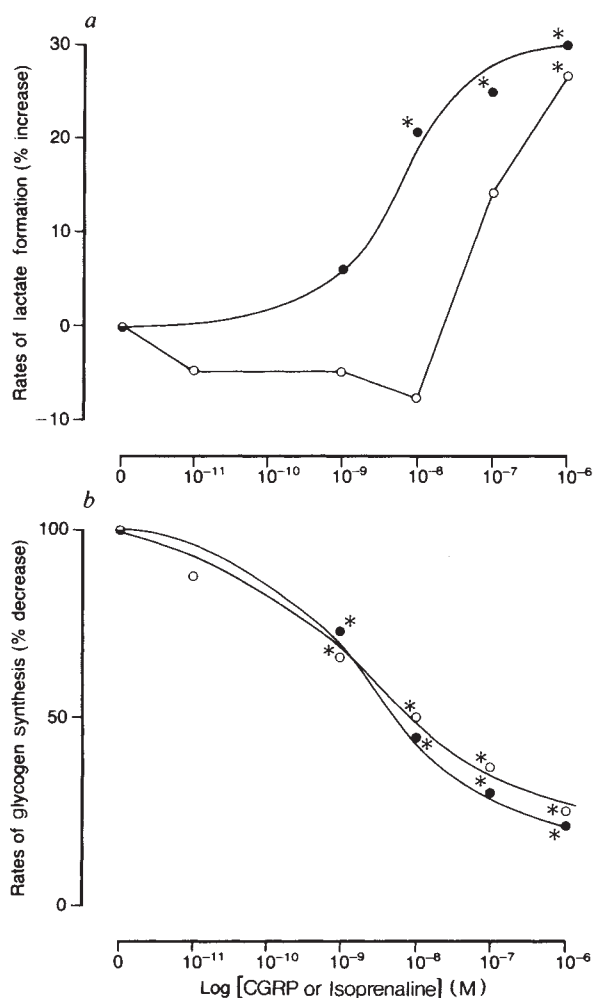


Fig. 1 Dose-related effects of rat calcitonin gene-related peptide-1 (CGRP-1) (Sigma) and isoprenaline on insulin-stimulated rates of lactate formation and glycogen synthesis in isolated incubated soleus muscle of the rat. Glucose concentration was 5.5 mM in all experiments. Comparisons of the effects of CGRP (○) and isoprenaline (●) on insulin-stimulated rates of *a*, glycolysis and *b*, glycogen synthesis. The values are presented as means of four separate experiments. Actual values of the rates of lactate formation and glycogen synthesis in the absence of isoprenaline or CGRP were 11.95 ± 0.76 and $3.18 \pm 0.23 \mu\text{mol h}^{-1} \text{g}^{-1}$ respectively. The standard error of the mean values, which were on average 7% of the mean values, are omitted for clarity. Statistically significant changes from control muscles are indicated by * (Student's *t*-test; $P < 0.05$).

Methods. Soleus muscle strips were prepared from 12-h fasted male Wistar rats (Harlan-Olac, Bicester; 160–180g) as previously described^{24,25}. The tendons of the muscles were ligated before attachment to stainless steel clips. Muscle strips were pre-incubated in Erlenmeyer flasks containing 3.5 ml Krebs-Ringer bicarbonate buffer, 7 mM N-2-hydroxyethyl-piperazine-N'-2-ethane-sulphonic acid, pH 7.4, and 5.5 mM pyruvate. Flasks were sealed and gassed continuously with O_2 and CO_2 in the ratio 19:1 (v/v). After pre-incubation of muscles in this medium for 30 min at 37 °C in an oscillating water bath, the muscle strips were transferred to similar vials containing identical medium (except without pyruvate) with added $[\text{U-}^{14}\text{C}]\text{glucose}$ ($0.5 \mu\text{Ci ml}^{-1}$) and insulin ($100 \mu\text{U ml}^{-1}$). The flasks were sealed and re-gassed for an initial 15 min in a 1-h incubation. At the end of the incubation period, muscles were blotted and rapidly frozen in liquid N_2 . The concentration of lactate in the incubation medium was determined spectrophotometrically¹⁷ and $[\text{U-}^{14}\text{C}]\text{glucose}$ incorporation into glycogen (glycogen synthesis) was measured¹⁷. One glucosyl unit indicates one glucose moiety incorporated into glycogen. Stock CGRP-1 solutions were prepared in 1% (w/v) gelatin. All results are presented in terms of g wet tissue.

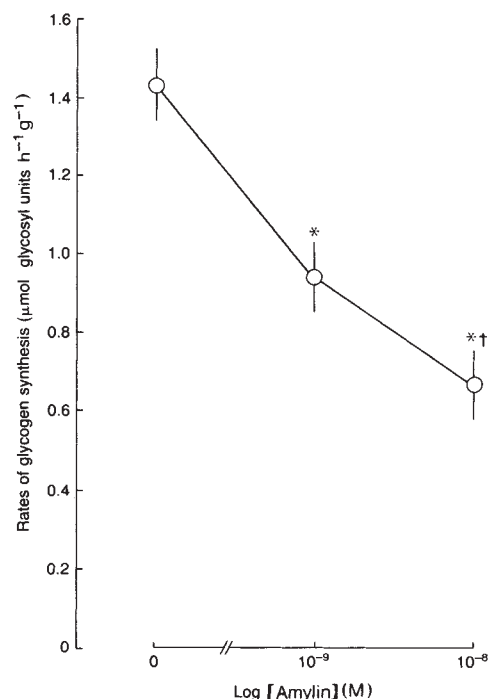


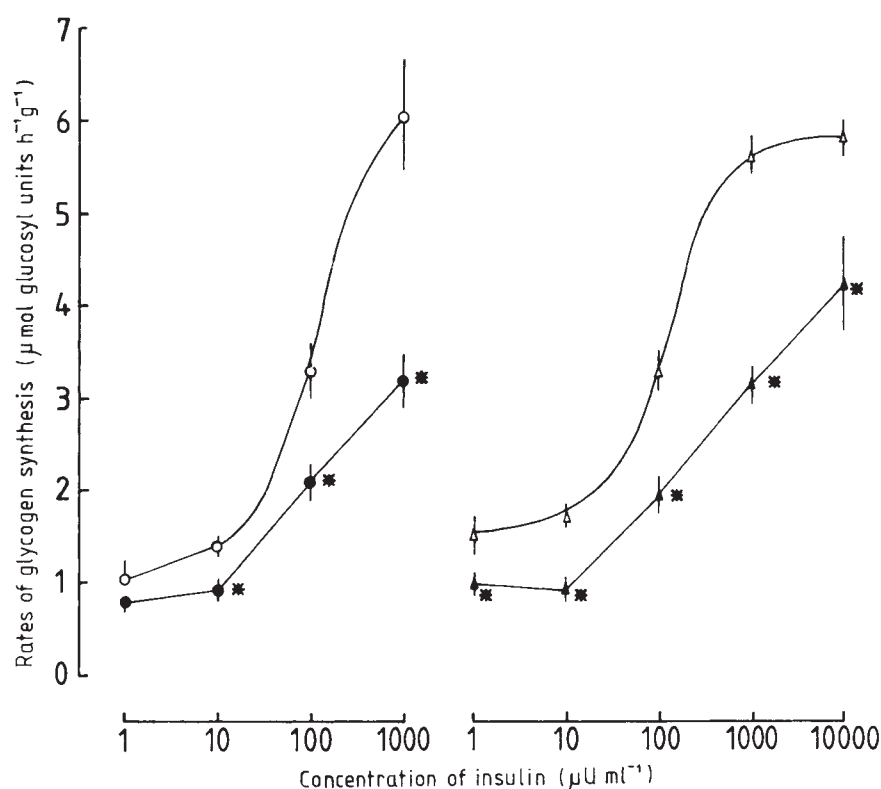
Fig. 2 Dose-related effects of pancreatic human amylin on insulin-stimulated rates of glycogen synthesis in isolated incubated stripped soleus muscle of the rat. The values are presented as means of at least four separate incubations. Statistically significant ($p < 0.05$, Student's *t*-test) decreases from control values are indicated by * (control against 10^{-9} M amylin); † (10^{-9} M amylin against 10^{-8} M amylin).

Methods. Muscle preparation and incubation methods were as described in Fig. 1 legend. Insulin causes rapid stimulation of glucose transport, glucose oxidation, glycolysis and glycogen synthesis in skeletal muscle and all these rates can be precisely measured in the incubated, stripped soleus muscle preparation of the rat¹⁷. Despite the multitude of factors that regulate the rate of glucose use in the whole body, very similar dose-responses of glucose disposal to insulin are obtained from both *in vivo* and *in vitro* muscle preparations. For example, under fasting conditions the EC_{50} values for insulin stimulation of glycogen synthase in the stripped soleus muscle of the rat *in vitro*²⁴ and in human muscle *in vivo*¹⁷ are 80 and $60 \mu\text{U ml}^{-1}$ respectively. Furthermore, the EC_{50} values for insulin stimulation of glucose transport in the intact soleus muscle preparation²⁴ is identical to that for soleus muscle *in vivo*²⁶. Typically, a submaximal concentration of insulin ($100 \mu\text{U ml}^{-1}$) stimulates the rates of glycolysis and glycogen synthesis, by ~50% and 125% above basal rates respectively.

tissues^{14–16}. Furthermore, this inhibition is characterized by decreased basal and maximal rates of glycogen synthesis and decreased sensitivity of this process to insulin (the insulin EC_{50} value is $\sim 350 \mu\text{U ml}^{-1}$; see Fig. 3). Similar responses are observed in skeletal muscle isolated from rats half-way through their feeding (0300h) and post-absorptive periods (1600h)¹⁷ (so insulin EC_{50} values for glycogen synthesis are 380 and $490 \mu\text{U ml}^{-1}$ respectively). The possibility that CGRP or amylin may cause, at least in part, the physiological insulin resistance that occurs during the dietary cycle warrants further investigation.

Type 2 diabetes is characterized primarily by a relative decrease in rates of insulin secretion from the pancreas and pathological insulin resistance in skeletal muscle, although incomplete glucose-induced suppression of hepatic glucose production may also be a factor¹⁸. The insensitivity of glucose disposal to insulin in skeletal muscle *in vivo* has been thoroughly investigated in studies with combined indirect calorimetry and the euglycaemic clamp (at high and low insulin concentrations)¹⁹. These studies demonstrate that the chief defect in

Fig. 3 Effects of insulin on the rates of glycogen synthesis in stripped soleus muscle of the rat incubated in the absence or presence of either human pancreatic amylin (10^{-9} M) or rat calcitonin gene-related peptide-1 (10^{-9} M). Results are presented as means $\pm$ standard errors of the means of at least three individual experiments. Statistically significant differences between (non-paired Student's *t*-test; $p < 0.05$) control muscles ($\circ, \Delta$) and muscles treated with either human amylin ($\bullet$) or rat CGRP-1 ($\blacktriangle$) are indicated by *. Methods for preparation and incubation of soleus muscles are as given in Fig. 1. legend.



insulin-resistant individuals is a decrease in the insulin-stimulated rates of non-oxidative glucose disposal (glycogen synthesis) in skeletal muscle²⁰.

Amylin is deposited as amyloid in the type 2 diabetic pancreas⁸ and has 46% amino acid sequence identity with CGRP-2 (ref. 8). Both peptides are 37 amino acids long, have nearly identical N- and C-terminal regions and an intramolecular disulphide bond between cysteine residues 2 and 7 (ref. 8). Amylin could contribute to decreased rates of both insulin secretion and glucose disposal as glycogen in muscle. The latter suggestion was investigated by extraction of pancreatic amylin from human diabetic pancreas as previously described⁸. It was purified further using two more cycles of reverse-phase HPLC, when it eluted as a single peak. Identity of the molecule was confirmed by N-terminal protein sequencing, using an Applied Biosystems 470 A protein sequencer²¹. No contaminating peptide sequence was detected. Quantitative amino acid analysis²² showed the

amylin was more than 90% pure. Similarly, the synthetic CGRP used was shown to be more than 95% pure on the basis of reverse-phase HPLC.

Amylin at two concentrations (10^{-9} and 10^{-8} M) significantly decreased the rates of basal glycogen synthesis (Fig. 2), but had no effect on rates of lactate formation (data not shown). This is the first demonstration of a biological activity of native amylin. Moreover, this is qualitatively the same response as that observed in insulin-resistant skeletal muscle of type 2 diabetics²⁰. As deposition of amylin as amyloid within the islets of Langerhans is characteristic of type 2 diabetes mellitus, there could be sufficiently high levels of amylin in the bloodstream of diabetics to interact with the CGRP effector system in muscle and so cause impaired glycogen metabolism, although this remains to be proved. These findings indicate that CGRP is a potent neuropeptide modulator of cellular glucose metabolism. The relative potency of pancreatic human amylin and rat CGRP-1

Table 1 Modulation of basal and insulin-stimulated rates of glycolysis and glycogenesis by CGRP-1

[CGRP-1](M)	[Insulin] ($\mu\text{U ml}^{-1}$)	Rates ($\mu\text{mol per h per g wet tissue}$)			
		0	10^{-10}	10^{-9}	10^{-8}
Lactate formation	1	8.09 ± 0.6 (10)	8.06 ± 0.9 (3)	8.55 ± 0.4 (3)	12.31 ± 0.5 (3)*
	10	8.36 ± 0.5 (13)	8.53 ± 0.4 (6)	7.78 ± 1.6 (3)	11.16 ± 0.5 (3)*
	100	11.07 ± 0.4 (12)	13.18 ± 1.2 (6)	11.15 ± 1.6 (3)	11.90 ± 0.5 (3)
	1,000	12.60 ± 0.8 (13)	13.61 ± 0.6 (6)	11.99 ± 0.7 (3)	14.07 ± 0.9 (3)
[^{14}C]glycogen synthesis	1	1.54 ± 0.2 (10)	0.99 ± 0.2 (3)	0.99 ± 0.1 (3)*	1.05 ± 0.2 (3)*
	10	1.72 ± 0.1 (13)	1.34 ± 0.2 (6)	0.92 ± 0.1 (3)*	1.35 ± 0.2 (3)*
	100	3.80 ± 0.2 (12)	2.84 ± 0.2 (6)*	1.94 ± 0.2 (3)*	1.75 ± 0.4 (3)*
	1,000	5.66 ± 0.2 (13)	4.94 ± 0.6 (6)	3.16 ± 0.2 (3)*	3.01 ± 0.4 (3)*
	10,000	5.81 ± 0.2 (3)	5.41 ± 0.4 (3)	4.24 ± 0.5 (3)*	3.01 ± 0.4 (3)*

The effects of insulin on the rates of formation of lactate and ^{14}C -labelled glycogen in the isolated incubated soleus muscle preparation of the rat in the presence and absence of CGRP-1. The rates of lactate production and glycogen synthesis were assessed as described in the Fig. 1 legend. Results are presented as the means ($\pm$ standard error of the means) for the number of observations given in parentheses. Differences statistically significant from untreated muscles are indicated by the asterisks ($P < 0.05$; Student's *t*-test). The number of individual experiments for each treatment condition are given in parenthesis.

is shown in Fig. 3. Amylin (10^{-9} M) and CGRP (10^{-9} M) significantly decreased rates of glycogen synthesis at basal ($10 \mu\text{U ml}^{-1}$), submaximal ($100 \mu\text{U ml}^{-1}$) and maximal ($1,000 \mu\text{U ml}^{-1}$) concentrations of insulin. Furthermore, the extent to which glycogen synthesis was inhibited was almost identical even though human amylin was incubated with rat muscle (see Fig. 3). These results, coupled with the observation that CGRP (ref. 23), and perhaps amylin, decreases the rate of insulin secretion both *in vivo* and *in vitro*, lead to the hypothesis

that an abnormality of amylin and/or CGRP homeostasis may underlie the pathogenesis of type 2 diabetes mellitus.

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A cytoskeletal spring in cochlear outer hair cells

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Normal hearing in mammals depends on an active mechanical filter, within the cochlea, which separates different sound frequencies before neural encoding. Experiments on the intact cochlea¹⁻⁴ indicate that the critical cellular components underlying the process are probably the outer hair cells^{5,6} which are strategically placed to influence movement of the basilar membrane. This idea is attractive because isolated cells can generate axial forces⁷ at acoustic frequencies⁸ when electrically stimulated. The mechanical properties of cells are largely determined by structures closely associated with the plasma membrane^{9,10}. We show here, using light and electron microscopy, that beneath this membrane lies a lattice of crosslinked circumferential filaments which are pitched at a mean angle of 15° to the transverse axis of the cell. The lattice is sufficient to retain the shape of the cell following demembration and mechanical deformation. The structure of the lattice allows it to be described as a coiled helical spring but with longitudinal stiffness primarily determined by the crosslinks. Direct measurements of longitudinal stiffness reported here indicate that the lattice contributes 5-10% of the stiffness. We propose that the 'circumferential lattice' ensures that outer hair cells can act as directed force generators within the organ of Corti, a prerequisite in current descriptions of cochlear micro-mechanics¹¹⁻¹³.

Isolated outer hair cells (OHCs) from the guinea pig organ of Corti are tubular, 30-100 μm long depending on their original location along the basilar membrane, and about 10 μm in diameter. Their shape can be maintained following substantial deformations of the cell, and this is probably achieved by elastic elements associated with the plasma membrane^{9,10}. When cells are demembrated with Triton X-100 in a buffer designed to stabilize cytoskeletal proteins¹⁴ (CSK buffer), they increase in length by up to 12%, but retain their shape (Fig. 1a,b). The CSK buffer without Triton X-100 has an osmotic strength of 600 mOs, almost twice the osmotic pressure inside OHCs, and

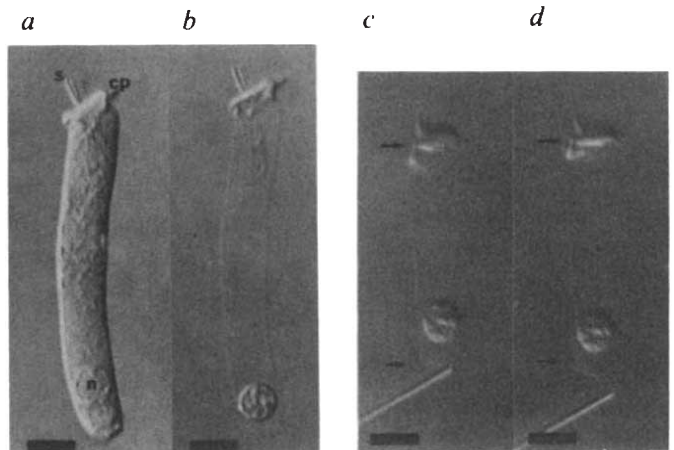


Fig. 1 An isolated OHC *a*, before and *b*, after 10 min in 0.5% Triton X-100 in a buffer designed to stabilize cytoskeletal proteins (CSK: 100 mM NaCl; 300 mM sucrose; 3 mM MgCl_2 ; 10 mM PIPES (pH 6.8), 1.2 mM phenylmethyl sulphonate)¹⁴. During demembration, the cell elongates by about 1 μm . The cell nucleus (n), cuticular plate (cp) and stereocilia (s) are clearly visible. A bundle of fibrous cytoplasmic structures project into the cell from the cuticular plate. *c*, A demembrated cell attached to the microscope slide only at the cuticular plate and compressed longitudinally by $\sim 2 \mu\text{m}$ using a glass probe. *d*, When the probe is removed the cell returns to its original length and diameter. Scale bars, 10 μm . In *c* and *d* the two arrows are 45 μm apart.

Methods. The organ of Corti was removed from the cochlea by microdissection and incubated for 20 min in cell culture medium (L-15, Gibco) containing 1 mg ml^{-1} trypsin (Sigma type IV)⁹. OHCs were then dissociated mechanically and observed in differential interference contrast on an inverted microscope (Zeiss IM). Experiments were conducted at room temperature. Stiffness measurements were made on cells isolated by mechanical dissociation only to protect exposed proteins from enzyme degradation. Glass fibres were etched from lead-free glass wool (BDH) to a final diameter of 1-2 μm . Fibre stiffness calibrated by measuring the vertical displacement produced by single acrylic spheres¹⁵. Fibres of a typical stiffness of about $100 \text{ pN } \mu\text{m}^{-1}$ were manipulated by a piezoelectric probe driven by a 5 Hz triangular signal, and monitored by a linear photodiode position sensor¹⁵⁻¹⁷; cell stiffness was then calculated from the reduction of the probe amplitude at cell contact during strains of $<3\%$.

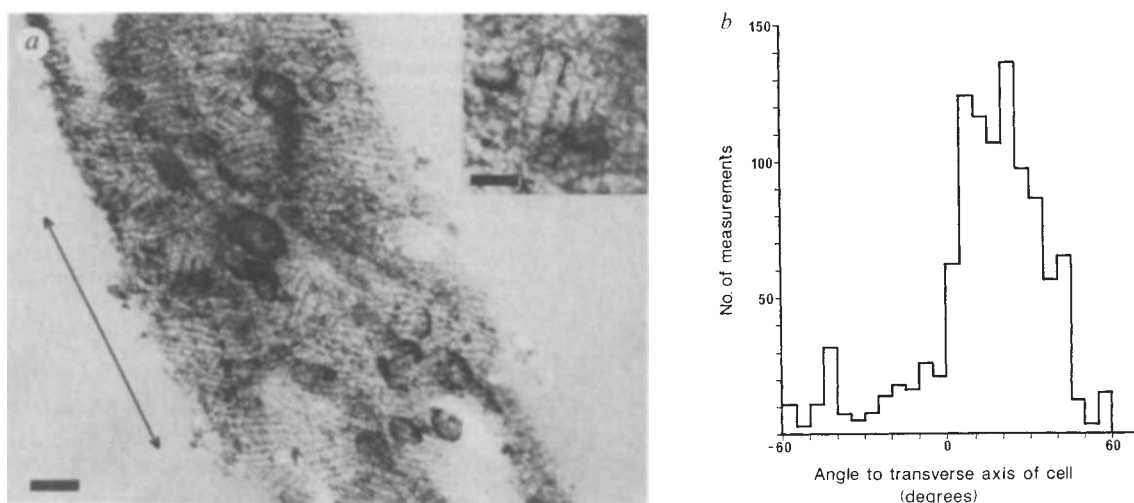


Fig. 2 *a*, Tangential thin section through the lateral boundary of a demembranated cell such as that shown in Fig. 1*b*; *b*, Orientation of circumferential filaments. *a*, Demembranation reveals a lattice composed of thicker, circumferentially-orientated filaments crosslinked by thinner filaments. Long arrow; longitudinal axis of the cell. Inset, two circumferential filaments (arrows) with crosslinks. The electron dense spots on the right-hand filaments are interpreted as pillars (see text). Scale bar, 200 nm; inset, 100 nm.

Methods. The cochlear spiral and attached organ of Corti were removed from the animal and incubated for 20 min in 0.5% Triton X-100 in CSK buffer. It was then fixed in 2.5% glutaraldehyde in the same solution, dehydrated, embedded in Epon and sectioned for transmission electron microscopy. The organization of the organ of Corti was not seriously disrupted by demembranation, and outer hair cells were readily identified by their location and by their cuticular plate. *b*, Histogram of the pitch of circumferential filaments in a sectioned cell, (downward angle between filament and the cell transverse axis). A square grid was placed over a $2\ \mu\text{m}^2$ area of a section to measure the orientation of filaments passing through each of 1,050 squares, each equivalent to an area of $44 \times 44\ \text{nm}$. Most (75%) of the measurements lie between 0 and 40° . Mean pitch, 15° ; angles plotted in 5° size classes.

when placed in it all OHCs collapse. When Triton X-100 is then added the OHCs are permeabilized and resume their original shape within 15–30 s. Demembranated cells also regain their diameter and length following displacements of the cuticular plate by several microns with a glass fibre (Fig. 1*c,d*). The cuticular plate with its associated fibrous projections and the nucleus are the only visible intracellular structures. These observations imply that elastic elements normally associated with the plasma membrane but insoluble in Triton X-100 can maintain cell shape following demembranation.

The lateral outline of demembranated cells appears in the transmission electron microscope to be composed of a regular circumferential filament lattice (Fig. 2*a*). This lattice is constructed of two types of filament: 'circumferential' filaments 5–8 nm in diameter; and crosslinks 2–3 nm in diameter. Measurements from thin sections grazing $2\text{--}6\ \mu\text{m}^2$ of the lattice show that 75–100% of the circumferential filaments are pitched at an angle of $0\text{--}40^\circ$ to the transverse axis of the cell (Fig. 2*b*). The mean pitch angle is 15° . Circumferential filaments are spaced at 40–50-nm intervals, and the longest filament measured was $1\ \mu\text{m}$. The crosslinks, interconnecting the circumferential filaments were 40–50 nm long and spaced irregularly at 12–25-nm intervals. Thin sections reveal no additional structures that might maintain the shape of a demembranated cell, suggesting that this function is fulfilled solely by the circumferential lattice. Electron-microscope images of whole demembranated cells in negative stain reveal a similar pattern of circumferential filaments throughout the cell.

A multi-layered system of intracellular membranes, the lateral cisternae, normally line the inner surface of the plasma membrane, and they are connected to it by an array of pillars^{10,18,19}. These pillars are arranged in rows in a pattern very similar to that of the circumferential filaments, and they could be represented in our sectioned material as electron-dense particles along the circumferential filaments. Previous structural evidence for a filamentous lattice interconnecting the pillars shows that such a lattice should lie adjacent to the lateral cisternae with the pillars projecting towards the plasma membrane¹⁹ (Fig. 3).

The protein composition of the circumferential filaments and crosslinks is unknown. Small amounts of actin have been located in the region of circumferential lattice in fixed cells²⁰, and rhodamine phalloidin, which binds selectively to polymerized (filamentous) actin, indicates the presence of this protein in the lattice of demembranated cells. A protein resembling spectrin has also been reported in the region of the lattice²¹. This result is of interest because spectrin is an important component of the mammalian erythrocyte cytoskeleton, which has elastic properties comparable with those of OHCs²².

From the structure it is possible to estimate the contribution of the circumferential filaments and crosslinks to the stiffness of each OHC. The filaments could establish the tubular shape of the cell by resisting changes in cell diameter, but they should permit limited shape changes because of their pitch angle. In fact their organization allows them to be modelled as system of simple helical springs. The force F required to compress a helical spring of coil diameter D , composed of N turns, by a distance x is given by²³

$$F = \frac{Gd^4}{8D^3N}x \quad (1)$$

where d is the diameter and G the modulus of rigidity of the circumferential element. If $d = 8\ \text{nm}$ and $G = 0.4\ \text{GPa}$, the value for collagen obtained from its Young's modulus²⁴, for a cell $L = 70\ \mu\text{m}$ long and $10\ \mu\text{m}$ in diameter, $N = 70/\pi \tan 15 = 8.32$. Thus, for the observed circumferential filament spacing, there should be about 330 springs in parallel. The contribution of the helical coils to the longitudinal stiffness is therefore $(dF/dx)_{\text{coils}} = 0.01\ \text{pN}\ \mu\text{m}^{-1}$.

This calculation indicates that the circumferential filaments alone cannot account for the longitudinal stiffness of the normal cell although they should provide circumferential reinforcement to oppose changes in diameter when the cell is compressed or inflated⁹. The crosslinks, however, are aligned quasi-longitudinally, and by linking adjacent coils they will tend to prevent longitudinal compression. The total stiffness of such an assembly

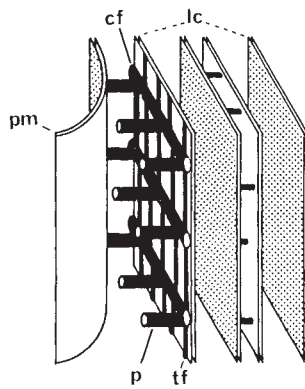


Fig. 3 Schematic diagram of the circumferential lattice. The plasma membrane is peeled away to show the pillars and their association with the circumferential filaments (cf) which are cross-linked by thin filaments (tf) and attached to the multilayered membranes of the lateral cisternae, (lc). The membranes of the lateral cisternae are interconnected by short filaments. Extracellular membrane (pm) surfaces and intraluminal surfaces of the lateral cisternae are shaded.

is given approximately by

$$(dF/dx)_{\text{crosslinks}} = EafM/L \quad (2)$$

where a is the total cross-sectional area of the thin filaments (here assumed to be 3 nm in diameter), M is the number of crosslinks around the cell circumference, E is their Young's modulus and f the fraction of fibres longitudinally aligned. Reasonable estimates are $E = 1 \text{ GPa}^{24}$, $M = 1,570$ and $f = 0.05$, from Fig. 2. Thus, this population would produce a longitudinal stiffness of about $8 \text{ nN } \mu\text{m}^{-1}$, five to six orders of magnitude greater than that of the unlinked circumferential filaments. This calculated stiffness of demembranated cells may be an overestimate because the crosslinks are neither rigid nor continuous, and they may have a Young's modulus less than that of collagen.

We measured the longitudinal stiffness of both intact and demembranated cells directly using a $1\text{-}\mu\text{m}$ diameter glass probe of known stiffness (Fig. 1)^{15,16}. We excluded cells that buckled under compression. We found that stiffness varies inversely with cell length consistent with a constant modulus of compressibility for the cell. Measured in cells varying in length from $53\text{--}75 \mu\text{m}$, the stiffness of intact cells is $544 \text{ pN } \mu\text{m}^{-1}$ ($n = 7$, range $147\text{--}1,078 \text{ pN } \mu\text{m}^{-1}$); in demembranated cells the mean stiffness is $34 \text{ pN } \mu\text{m}^{-1}$ ($n = 6$, range $18\text{--}67 \text{ pN } \mu\text{m}^{-1}$), within the range of spring stiffness predicted above.

The cell membranes should account for some of the difference between intact and demembranated cells, by linking elements of the lattice together. Intact cells are under a small positive internal-fluid pressure⁹, arising from forces in the membrane, which should increase their stiffness further. The simplest explanation for cell elongation during demembranation is that the circumferential lattice in the intact cell behaves like a spring which is longitudinally compressed by the effect of tension in the cell cortex. The lattice can thus provide a passive antagonistic force to oppose active cell-length changes.

Several models of cochlear function require active elements (likely to be ensembles of about 100 OHCs) which oppose the dissipative, viscous damping of the basilar membrane motion^{6,12,13}. The inferred OHC forces are probably too small to affect the static compliance of the basilar membrane^{25,26}, but are of about the right magnitude to provide the requisite dynamic control of the basilar membrane mechanics.

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Cholinergic phenotype developed by noradrenergic sympathetic neurons after innervation of a novel cholinergic target *in vivo*

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Mammalian sympathetic neurons *in vivo* may express either a noradrenergic or cholinergic phenotype. In view of the opposing effect of noradrenaline and acetylcholine on most autonomic target organs, the target-appropriate expression of neurotransmitter is critical. We have examined the maturation of the sympathetic innervation of rat sweat glands to define the developmental mechanisms regulating neurotransmitter choice *in vivo*^{1–5}. Eccrine sweat glands and their sympathetic innervation develop together postnatally in the rat. Early postnatal innervation expresses only noradrenergic properties, but as the glands and their innervation mature, noradrenergic properties decrease dramatically and cholinergic features appear in the same population of neurons. To investigate the role of the sweat gland in this change we have used a transplantation paradigm which allows sweat glands to be innervated by sympathetic neurons that would normally innervate noradrenergic target organs and remain noradrenergic throughout life. We observe that the sympathetic neurons that innervate the novel cholinergic target alter their neurotransmitter properties and develop a cholinergic phenotype. These results indicate that target organs are able to induce appropriate neurotransmitter traits in the neurons that innervate them.

The development of a cholinergic phenotype in the sympathetic innervation of the sweat glands is characterized by production of acetylcholinesterase (AChE), choline acetyltransferase (ChAT, the synthetic enzyme for acetylcholine), and by cholinergic transmission^{1,3,5}. The sweat gland innervation contrasts with other sympathetic projections described in the rat; for example, sympathetic neurons that innervate the iris, heart and pineal gland retain noradrenergic characteristics throughout development⁶. One obvious difference among these populations besides neurotransmitter expression is the target organs they innervate.

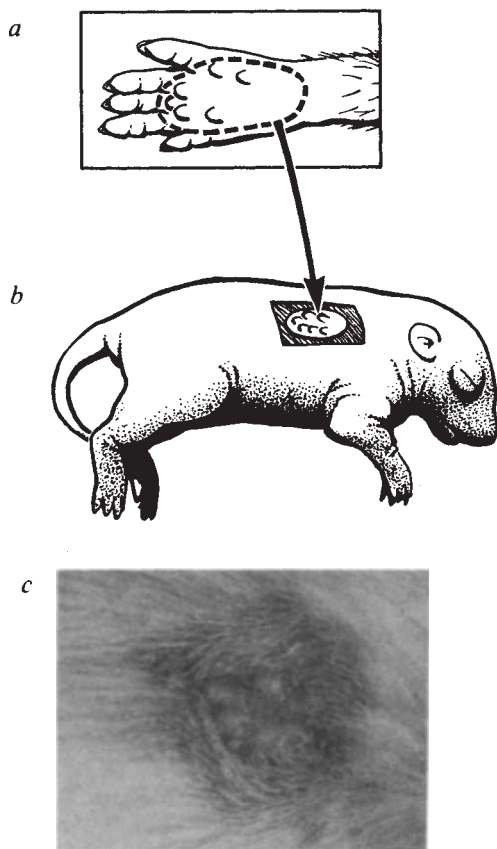


Fig. 1 Illustration of transplantation procedure. *a*, Footpad skin, including the epidermis, dermis and developing sweat glands, was removed from the ventral surface of donor rat feet. The dashed line indicates the boundaries of skin removed for the graft. Donor rats were typically 6–8 days old. *b*, The graft bed was prepared by removal of hairy skin in the right lateral thorax down to, but not through, the underlying panniculus carnosus. Following placement of the graft on the graft bed, the area was dressed and bandaged^{15,16}. Recipient rats were 3–8 days old. *c*, Photograph of footpad skin transplant at four weeks post-transplantation, $\times 1.5$. Note that the gross morphology of the transplant was maintained in the presence of elevated pads and absence of hairs. About two-thirds of the transplantations were successful in terms of graft survival. Lewis inbred rats were used in all experiments.

To investigate the role of the sweat glands in the cholinergic differentiation of their innervation, we have used a transplantation paradigm which takes advantage of the topographical segregation of noradrenergic and cholinergic sympathetic targets in rat skin (Fig. 1). Hairy skin normally receives noradrenergic sympathetic innervation, predominantly associated with piloerector muscles, but not cholinergic sympathetic innervation (see below). In contrast, the sweat gland containing glabrous skin of the footpads normally receives cholinergic sympathetic innervation^{1,3}. By transplantation of sweat gland-containing footpad skin to the lateral thorax of neonatal Lewis inbred rats, sympathetic neurons that would normally innervate noradrenergic targets in the hairy skin innervate sweat glands instead. The effects of target alteration on the expression of neurotransmitter-related traits were assayed by following the development of catecholamine histofluorescence, AChE staining and ChAT activity in the transplants.

A striking change in catecholamine histofluorescence was observed in the sweat-gland innervation over time (Fig. 2). Catecholamine-containing fibres first invaded the transplanted sweat glands at two weeks post-transplantation and formed an intensely fluorescent plexus in the glands by three weeks. Over the next few weeks the fluorescence intensity of the

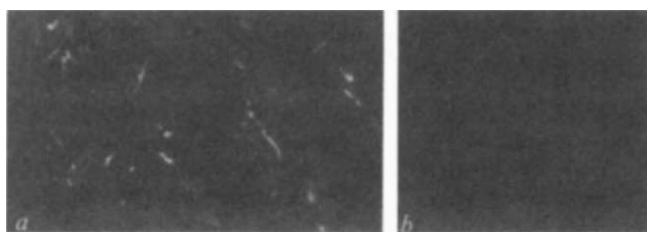


Fig. 2 Development of catecholamine histofluorescence in the innervation of the transplanted sweat glands. *a*, At two weeks post-transplantation, a plexus of brightly fluorescent fibres surrounded the transplanted sweat glands. *b*, At six weeks post-transplantation only occasional, faintly fluorescent fibres were observed.

Methods. Endogenous catecholamines were localized in 15- μ m cryostat sections of transplanted footpad with the glyoxylic acid technique¹⁷. $\times 130$.

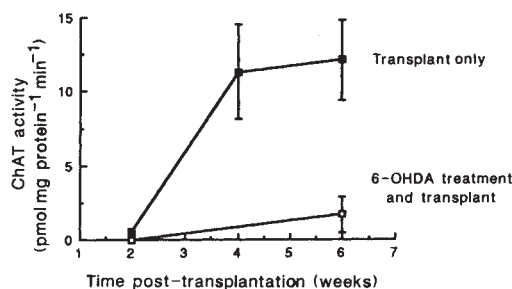


Fig. 3 Development of choline acetyltransferase activity in the transplanted footpads (closed squares). At two weeks post-transplantation, negligible ChAT activity was present. Between two and four weeks post-transplantation, there was a dramatic rise in ChAT activity in the transplants. The increased level of ChAT activity was maintained at six weeks post-transplantation. Some animals were treated with 6-hydroxydopamine (6-OHDA) before receiving transplants (open squares). Drug treatment inhibited the increase in ChAT activity seen at six weeks post-transplantation. Each point represents the mean $\pm$ s.e.m. of activities from at least three separate animals.

Methods. ChAT activity was measured in homogenates of transplanted footpads using modifications³ of the method of Fonnum¹⁸. The value expressed is the activity of the homogenates that was inhibited by 500 μ M naphthylvinylpyridine, a specific inhibitor of ChAT. In most experiments, the radiolabelled product acetylcholine was isolated by ion-pair extraction as described previously, but in other experiments acetylcholine was isolated by thin layer chromatography. Briefly, after the addition of carrier acetylcholine to the incubation tubes, 10 μ l of assay mixture was spotted on a Kodak cellulose chromatogram sheet which was run until the solvent (1-butanol:acetic acid:water, 90:3:18 by volume) front had travelled 10 cm. The ACh band was visualized using either ultraviolet light or iodine vapour, then cut out and extracted with 1 ml of 0.5 mg ml⁻¹ zinc chloride solution in a scintillation vial; scintillation fluid was added and the sample counted. In both control and transplanted footpads, activities measured by the two isolation procedures agreed within $\sim 10\%$. 6-hydroxydopamine treatment was performed as described previously², except that the daily dose of drug was decreased to 50 mg kg⁻¹. Drug-treated neonates typically received transplants on postnatal days 7–10.

catecholamine-containing fibres decreased and, by six weeks, only occasional, faintly fluorescent, fibres were observed. The decrease in fluorescence intensity in the sweat gland innervation contrasted with the catecholamine-containing fibres in control hairy skin which remained brightly fluorescent throughout this time. It is important to note that fibres associated with blood vessels in the transplant also remained brightly fluorescent throughout the experimental time course.

ChAT activity in the transplanted footpads increased dramatically following transplantation (Fig. 3). Negligible ChAT activity was present at two weeks post-transplantation but

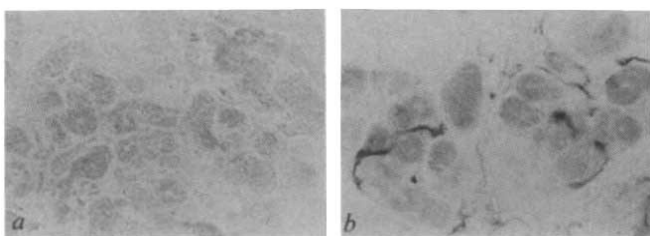


Fig. 4 Development of acetylcholinesterase staining in the innervation of the transplanted sweat glands. *a*, At two weeks post-transplantation, no AChE-staining fibres were associated with the sweat glands. *b*, At six weeks post-transplantation many AChE-positive fibres coil around the sweat glands.

Methods. Acetylcholinesterase staining was examined in 15-micron cryostat sections of transplanted footpad¹, using 4% paraformaldehyde as fixative. AChE staining in a section adjacent to *b* and in about half the transplants was completely inhibited by the addition of 100 μ M 1,5-bis(4-allyl-dimethylammonium-phenyl) pentan-3-one-diiodide (BW284C51, Burroughs Wellcome), a specific inhibitor of AChE. In other transplants a few fibres still stain in the presence of the inhibitor. The BW284C51-insensitive staining could represent nonspecific esterase in sensory neurons because there is normally a transient expression of non-specific esterase staining in some sensory fibres in control hairy skin, also because this staining is sometimes seen in animals treated with 6-hydroxydopamine before transplantation. $\times 130$.

between two and four weeks there was an approximately 20-fold rise in ChAT activity in the transplanted footpads. Activity was maintained at six weeks. ChAT activity was not detectable in control hairy skin at any time during development.

AChE staining also appeared during development of the sweat gland innervation (Fig. 4). Unlike the expression of catecholamines, AChE staining was not observed in the innervation of the transplanted sweat glands until three weeks post-transplantation, when it was only faint, but by four weeks it had greatly increased in intensity in the fibres surrounding the transplanted sweat glands; this level was maintained at six weeks. At no time in development was AChE staining detected in the innervation of control hairy skin.

To determine whether the observed changes in neurotransmitter-related properties occurred in sympathetic neurons, neonatal rats were treated with 6-hydroxydopamine (6-OHDA) before receiving transplants (see Fig. 3). Administration of 6-OHDA to neonates results in almost-total sympathectomy, presumably as a result of chemical axotomy and deprivation of nerve growth factor^{7,8}. As expected, drug treatment greatly decreased the number of catecholamine-containing fibres innervating the transplant. 6-OHDA administration also dramatically inhibited the development of both AChE staining and ChAT activity in the transplants (Fig. 3). This indicates that the neurons responsible for the increase in cholinergic properties in the transplants were sensitive to 6-OHDA shortly after birth of the animal.

To demonstrate that the transplantation procedure itself was not responsible for the changes in neurotransmitter properties, we performed transplantations in which hairy skin was replaced with hairy skin from a donor animal, rather than with footpad skin. This procedure did not elicit the changes in catecholamine fluorescence, AChE staining or ChAT activity (0.7 ± 0.6 pmol mg protein⁻¹ min⁻¹ at six weeks post-transplantation) seen with the footpad transplants.

Our results indicate that a cholinergic sympathetic target, the sweat gland, promotes cholinergic differentiation in a population of sympathetic neurons that would normally continue to undergo noradrenergic differentiation. This is evidence that postnatal sympathetic neurons *in vivo* are not irreversibly committed to a particular neurotransmitter phenotype, but are able to alter their phenotypes in response to a novel environment. Preliminary retrograde tracing studies show that the sympathetic fibres that innervate the transplant arise from cell bodies in the

mid-thoracic ganglia (usually T5–T8); these ganglia do not normally provide innervation to sweat glands. Neurotransmitter plasticity has been demonstrated in cell culture, in which noradrenergic neurons isolated from neonatal sympathetic ganglia can acquire cholinergic function with appropriate manipulation of the growth conditions^{9–11}. An alternative explanation for our findings is that the transplanted sweat glands cause the selective survival of a population of cholinergic sympathetic neurons that would normally die during development, but as we have no evidence for such a population in either developing or mature control hairy skin, this is unlikely.

The factors responsible for the adrenergic to cholinergic switch in the innervation of the rat eccrine sweat gland are unknown, but comparison with cell-culture studies would suggest that soluble target-derived proteins could be important^{12–14}.

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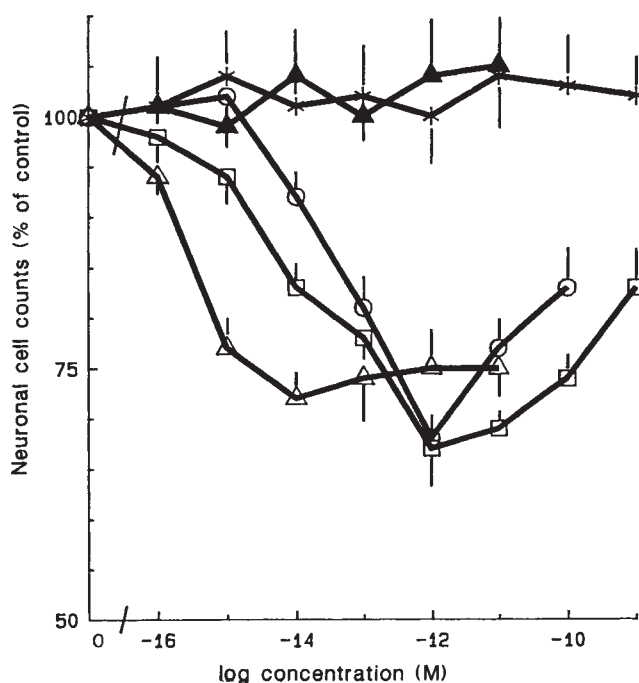
Neuronal cell killing by the envelope protein of HIV and its prevention by vasoactive intestinal peptide

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The clinical manifestations of AIDS (acquired immune deficiency syndrome) often include neuropsychiatric and neurological deficits^{1–4}, including early memory loss and progressive dementia. HIV (human immunodeficiency virus), the aetiological agent of AIDS, is probably carried by infected macrophages in the central nervous system^{5–7}. The virus enters cells by binding its envelope glycoprotein gp120 to the CD4 antigen^{8–10} present on brain and immune cells^{11–13}. From the data reported in this paper, we now suggest that the neuronal deficits associated with HIV may not be entirely a result of infectivity, but that gp120 shed from HIV^{14–16} could directly produce the neuropathology as a result of its interference with endogenous neurotrophic substances. It is known that an analogue of a sequence contained in vasoactive intestinal peptide

Fig. 1 HIV envelope protein causes neuronal cell death in hippocampal cultures. Recombinant gp120 (open triangles) from the LAV encoding sequences was compared to native gp120 purified³⁸ from two natural isolates, the 3B (circles) and RF2 (squares). A recombinant gp160 (asterisks) from the 3B coding sequences was expressed in *Spodoptera frugiperda* cells with a recombinant baculovirus³⁹. The gp160 was partially purified³⁹ so that the preparation contained three major bands, including one at a relative molecular mass (M_r) of 160,000 (160K). The recombinant gp120 was produced by recombinant vaccinia virus containing LAV envelope encoding sequences from amino acids 1–543 (that is, it includes the entire gp120 sequence plus 20 amino acids downstream from the proteolytic cleavage site). The glycoprotein was purified from serum-free medium from recombinant, virus-infected cells using lentil lectin affinity chromatography. This preparation was >85% pure, with a main single band of about 120K. Control medium was prepared in the same manner from cells infected with vaccinia virus without the gp120 construct (closed triangles). The various gp120 preparations were incubated with one-week-old dissociated hippocampal cultures for five days. Neurons were then identified immunocytochemically²¹ with antisera to neuron-specific enolase. Neurons were counted in 100 fields at predetermined stage coordinates without knowledge of treatment. The total area counted was 50 mm². For the 3B isolate, each value is the mean of six dishes from two separate experiments. The RF2 and gp160 values are the mean from four dishes. Error bars indicate the standard error of the mean. Significant decreases ($P < 0.01$) from controls were observed at concentrations greater than 10^{-14} M for both isolates. All statistical comparisons were by analysis of variance with Student-Keuls multiple comparison of means. Control cultures from these experiments had neuron counts that ranged from 750 to 1,000 cells per 50 mm² with standard errors that were <4% of the mean. The dissociated hippocampal cultures⁴⁰ were prepared from 16–17-day-old embryos (C57B1/6 mice), and plated at low density (50,000 cells per 35-mm dish) on to confluent layers of astrocytes. The astrocyte feeder layers were prepared from the hippocampi of newborn mice. Following treatment with 0.125% trypsin for 15 min, the tissue was triturated and plated at 2.5×10^5 cells into 35-mm tissue culture dishes coated with Vitrogen (Collagen, Palo Alto) and poly-L-lysine (M_30 –70K 10^{-5} M in 0.15 M borate, pH 8.4, Sigma). The resulting feeder layers were grown in Eagle's minimal essential medium (MEM, formula 82–0234, Gibco) with 10% fetal bovine serum until confluent (12–14 days); feeder layers prepared in this way were devoid of neurons and consisted predominantly of flat cells that were stained by antibodies to glia fibrillary acidic protein. When hippocampal neurons were added to the confluent feeder cultures, the medium was changed to 94% MEM, 5% horse serum (Gibco) and an added nutrient supplement containing insulin, transferrin, putrescine, selenium, corticosterone, progesterone and triiodothyronine²¹. Hippocampal cultures were treated one day after plating with 5'-fluoro-2'-deoxyuridine ($15 \mu\text{g ml}^{-1}$ plus uridine, $35 \mu\text{g ml}^{-1}$) to suppress overgrowth of background cells. The neuronal cell cultures were allowed to grow for one week before the beginning of the experimental test period. Before treating with gp120, the cultures were given a complete change of medium.



(VIP) occurs in all known sequenced gp120 isolates^{17–20} and that VIP is important for neuronal survival in cell culture^{21–23}. Here we show that purified gp120 from two diverse HIV isolates and a recombinant gp120 from a third isolate were all potent in specifically producing significant neuronal cell death in dissociated hippocampal cultures derived from fetal mice, and that this could be reduced by monoclonal antibodies against the murine²⁴ CD4 antigen and completely antagonized by VIP.

Cells from mice do not seem to become infected when exposed to HIV, but it is not clear whether this is due to the inability of gp120 to bind to the mouse CD4 antigen or a more distal mechanism related to viral entry or replication. The use of hippocampal cultures has allowed testing for a pathological interaction other than infectivity between murine cells and gp120. This model system gives high resolution for neuron counting as well as analysis of a complex system with interacting cell types found in the brain. At the culture age examined in our studies, the neurons exhibit functional synapses and neuron-specific enolase immunoreactivity.

Figure 1 shows that incubation of hippocampal cultures with gp120 purified from either of two HIV isolates, 3B and RF2, produced nearly identical and significant neuronal cell losses at extraordinarily low concentrations. The dose-response curves reveal the consistent attenuation of neuronal cell loss with concentrations of gp120 $> 10^{-12}$ M. Time-course experiments of the neuronal deficits produced by gp120 revealed a slow, progressive effect to a maximal observed loss of $51 \pm 4\%$ of control counts after three weeks of treatment. Recombinant gp120 produced from the encoding sequences of the LAV strain of HIV in a vaccinia virus vector also decreased neuronal survival when

tested under the same experimental conditions, whereas medium prepared and diluted identically from the parent cells infected with vaccinia virus without the gp120 construct had no detectable effect (Fig. 1). A recombinant gp160, expressed from the 3B-strain sequence in an insect cell, had no detectable effect on neuronal survival (Fig. 1), possibly because its tertiary structure is different from that of gp120 because of differences in glycosylation and/or the presence of the gp41 sequence.

Peptide-receptor interactions often display biphasic responses similar to those in Fig. 1 and previous studies with VIP_{10–28}, a putative VIP antagonist, showed that the dose-response curve for neuronal cell death in dissociated spinal cord neurons was V-shaped²¹. The reasons for the biphasic nature of these responses are not clear, but they may include one or more of the following: partial agonist/antagonist properties, activation of another receptor, down-regulation of receptors at high doses, or ligand self-association at high concentration.

To determine whether mouse CD4 surface antigen is involved in the neuronal deficits produced by gp120, monoclonal antibodies directed against CD4 were added to hippocampal cultures treated with concentrations of gp120 that produced the greatest amount of neuronal cell death. The concentration-effect curve for one of these antibodies (GK1.5; refs 25 and 26) is shown in Fig. 2. This rat IgG2b antibody gave concentration-dependent protection against the neuronal cell death produced by gp120 (3B strain). Thus, a monoclonal antibody that inhibits the functional activity (proliferation and lymphokine release of mouse T cells) also effectively blocked the neurotoxic action of gp120. In addition, a rat IgM monoclonal antibody to mouse CD4, RL172.4 (ref. 27), also prevented gp120-induced neuronal

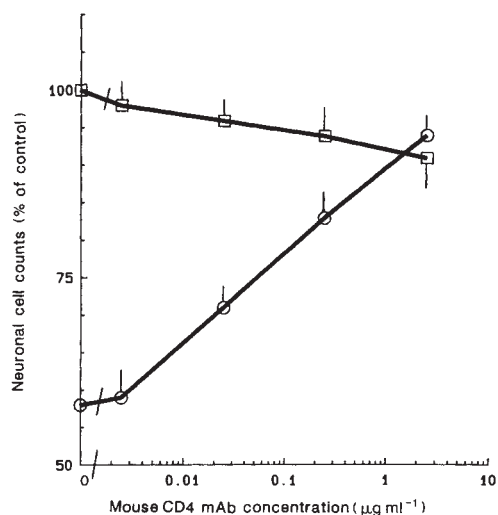


Fig. 2 A monoclonal antibody (GK1.5) against the murine CD4 receptor reduces gp120-induced neuronal cell death in mouse hippocampal cultures. GK1.5, a rat IgG2b monoclonal antibody^{25,26}, was affinity-purified on a goat anti-rat kappa-Sepharose column (Zymed, California), dialysed against phosphate buffered saline, subjected to an enzyme-linked immunosorbent assay analysis for quantitation, and then added to hippocampal neurons after one week in culture. A comparison was made between cultures treated with antibody alone (squares) and antibody plus 10^{-12} M gp120 from the 3B isolate (circles). The gp120 and antibody were added at the same time. Significant increases ($P < 0.01$) in neuron cell counts were observed with 0.02 – $2.5 \mu\text{g ml}^{-1}$ of GK1.5 compared to those in cultures with gp120 only. Treatment with GK1.5 alone did not produce a significant change in neuron counts compared with control cultures. Each value is the mean of five dishes from two experiments. Error bars indicate standard error of the mean. Control cultures had $1,100$ – $1,300$ cells per 50 mm^2 with standard errors for each experiment being $< 4\%$ of the mean. Monoclonal antibody, mAb.

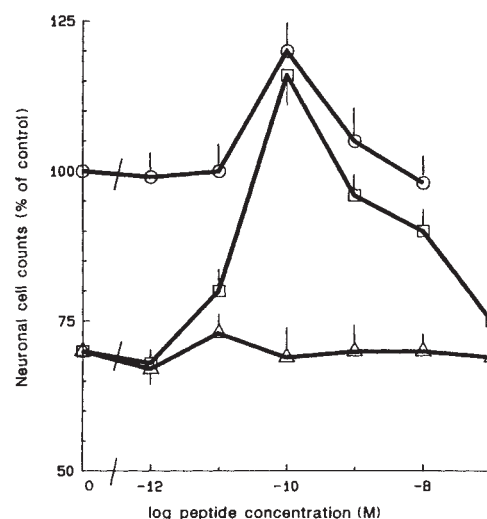


Fig. 3 Vasoactive intestinal peptide prevents gp120-induced neuronal cell death in hippocampal cultures. One-week-old cultures were treated for five days with VIP alone (circles), VIP plus 10^{-12} M gp120 (squares), or secretin plus gp120 (triangles). Peptides were obtained from Peninsula Lab. (Belmont, California). Each value is the mean of 4–6 dishes from two experiments. Error bars show standard error of the mean. In comparison to cultures treated with gp120 alone, there were significant increases ($P < 0.02$) in the number of neurons with the addition of 10^{-10} M to 10^{-8} M VIP plus gp120. Treatment with 10^{-10} M VIP plus gp120 resulted in neuronal cell counts that were not different from cultures treated with 10^{-10} M VIP alone. The control cultures from these experiments ranged from 850 to 950 neurons per 50 mm^2 with standard errors $< 4\%$.

cell death. The neuron counts of cultures treated with affinity-purified RL172.4 antibody ($0.25 \mu\text{g ml}^{-1}$) plus 1 pM gp120 were not significantly different from those of control cultures (data not shown). Neither a rat IgG2b (331.12, an anti-mouse IgM) nor a rat IgM (RA3-3A1, an anti-B220) control monoclonal antibody had any effect on preventing gp120-induced neuronal cell death when tested at similar concentrations as GK1.5 or RL172.4 (ref. 28 and data not shown). An additional control antibody to a neutral molecule located on neuronal and some non-neuronal cell surfaces²⁹ was also tested. A mouse IgG monoclonal antibody (A2B5) to surface GQ gangliosides was added ($20 \mu\text{g ml}^{-1}$) to gp120-treated cultures. The A2B5 control antibody did not prevent gp120-induced death and the antibody had no effect on neuronal survival when added to cultures without gp120. These results indicate that the neuronal cell death associated with gp120 treatment is a consequence of an interaction with the cell-surface mouse antigen CD4 or with a receptor that shares epitopes with it.

VIP, a neuropeptide present in brain³⁰ and immune³¹ cells, has been shown to have a neurotrophic action on neuronal survival in cell culture^{21–23}, and we therefore tested its effect on the neuron-depleting action of gp120. As shown in Fig. 3, neuron counts from gp120-treated cultures to which 10^{-10} M VIP had been added were not significantly different from those in cultures treated with VIP alone: VIP prevented the toxic action of gp120. Significant increases ($P < 0.001$) in the number of neurons surviving were observed with VIP treatment alone compared with untreated controls, consistent with a neurotrophic role for VIP in developing hippocampal cultures. Secretin, a peptide with significant sequence homology²² and size similarity to VIP, had no influence on gp120-induced neuronal cell death (Fig. 3). At

concentrations greater than 10^{-10} M, the survival-promoting activity of VIP was attenuated.

The mechanism by which VIP protects neuronal cells is not identified by this study. On the basis of sequence similarity, chemotactic bioassays, competitive binding studies and similarities of CD4 and VIP receptor distributions in monkey and human brain^{12,17–20,32}, it has been proposed that VIP and gp120 may compete for the same or similar receptors. Alternatively, gp120 might interfere with growth factors that are released or stimulated by VIP receptors on non-neuronal cells²³. There are other bioactive regions of gp120 with affinity for other receptors^{33–35} one of which (neuroleukin) has an effect on neuronal survival³³. We cannot exclude the possibility that multiple or interacting sites and substances mediate these effects on survival. Our studies demonstrate that gp120 produces neuronal death in hippocampal cultures that have established synaptic connectivity and that death can be prevented by monoclonal antibodies to mouse CD4 as well as the neuropeptide VIP. Peptide T, a substance with sequence similarity to VIP, has also been shown to be active in the survival assay described here. Peptide T, Thr-Thr-Ser-Tyr-Thr and Asp-Thr-Ser-Tyr-Gly, and other peptide T analogues^{19,20} can prevent gp120-induced neuronal cell death at low (10^{-10} M) concentrations³⁶. Anti-serum to peptide T (ARV isolate) prevents neuronal cell death produced by the 3B isolate of gp120³⁶, indicating that peptide T is important in the action of gp120.

If our *in vitro* results relate to the maintenance of neuronal survival *in vivo*, then they may explain a common neuropathological finding in AIDS^{5–7}. Gp120 secreted from infected subcortical macrophages may diffuse to act at a distance causing cortical abnormalities without obvious cortical infection, particularly given the small concentrations of gp120 which cause neuronal death. Our experimental observations were made in a developing neuronal system; the neuronal vulnerability to the toxic effects of gp120 may therefore be particularly relevant to

infants with AIDS, where the neurological effects are so severe^{2,37}.

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Interleukin-4 mediates CD8 induction on human CD4⁺ T-cell clones

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CD4 and CD8 antigens are simultaneously expressed on most of the cortical thymocytes, that weakly express the T-cell antigen receptor (TCR)/CD3 complex^{1,2}. Mature peripheral T cells, however, strongly express the TCR complex and are positive for either CD4 or CD8. Nevertheless, a small percentage of peripheral CD3⁺ T cells express CD4 and CD8 simultaneously³. These mature, double positive cells could be intermediates between CD4⁺CD8⁺ thymocytes and mature, single positive T cells, or they may originate from single positive T cells that acquire either CD4 or CD8. Here we report that activation and culturing of cloned CD4⁺ T cells in interleukin-4 (IL-4), results in the acquisition of CD8 due to its *de novo* synthesis. The IL-4-induced co-expression of CD8 on CD4⁺ T cells is reversible, in that CD8 disappeared from double positive T-cell clones isolated in IL-4, when they were cultured in IL-2. CD8 induced by IL-4 can be functional as a monoclonal antibody to CD8 inhibited anti-CD3-mediated cytotoxicity by a double positive T-cell clone.

Recently, we demonstrated that recombinant human IL-4 can promote the growth of T cells⁴. In experiments designed to test the effects of recombinant IL-4 on CD4⁺CD8⁺ T cells purified by sorting with a fluorescence-activated cell sorter, repetitive activation of CD4⁺ T cells in the presence of recombinant IL-4 resulted in the appearance of CD4⁺CD8⁺ T cells in the fraction which was initially CD4⁺CD8⁻. In contrast, CD8⁺CD4⁺ cells activated and cultured in IL-4 remained positive for CD8 only (results not shown). These results indicated that IL-4 can induce CD8 on activated CD4⁺ T cells. However, for maximal induction of CD8, weekly stimulation of the CD4⁺ cells for a period of three weeks was necessary. Therefore, it was possible that IL-4 induced the selective outgrowth of a very small, initially undetectable, percentage of double positive lymphocytes present in the starting populations. To confirm that IL-4 induces CD8 on individual CD4⁺ T cells, a cloning experiment was carried out with IL-4 or with IL-2 (for comparison), as growth factors.

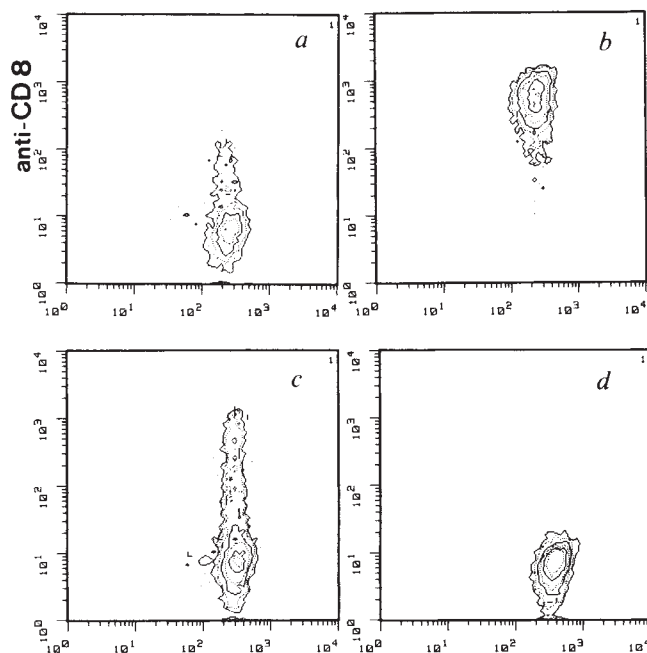
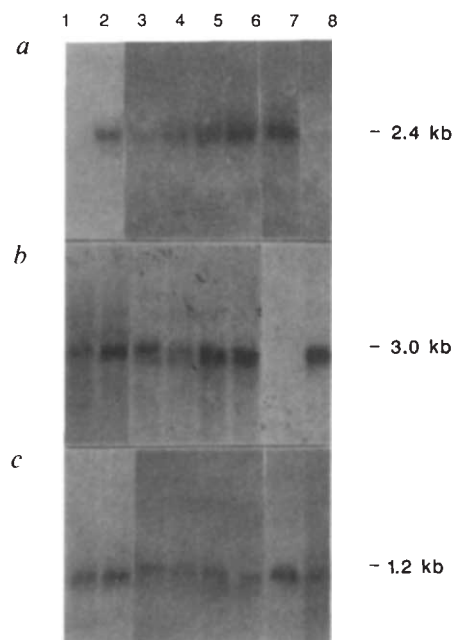


Fig. 1 Changes in phenotype of a CD4⁺CD8⁺ T-cell clone obtained in IL-4 and cultured in IL-2 (a) or maintained in IL-4 (b) and of a CD4⁺CD8⁻ T-cell clone isolated in IL-2 and subsequently cultured in IL-4 (c) or maintained in IL-2 (d). Log green fluorescence using fluorescein isothiocyanate (FITC)-labelled anti-CD4 is shown on the abscissa and the log red fluorescence using phycoerythrin (PE)-labelled anti-CD8 on the ordinate.

Methods. The IL-4-isolated CD4⁺CD8⁺ T-cell clone CD-129 and the IL-2-isolated CD4⁺CD8⁻ T-cell clone CD-206 were cultured for 21 days in either IL-4 (200 U ml⁻¹) or IL-2 (20 U ml⁻¹). The clones were stimulated weekly at a concentration of 2×10^5 cells ml⁻¹ with feeder cells consisting of irradiated (4,000 rad) allogeneic PBL (10^6 cells ml⁻¹), irradiated (5,000 rad) JY cells (10^5 cells ml⁻¹) and 0.1 μ g ml⁻¹ PHA (Wellcome) supplemented with the appropriate lymphokine. Cytofluorographic analysis was performed six days after re-stimulation with feeder cells. At that time, the feeder cells were completely disintegrated. The cells were washed and incubated with the FITC-labelled anti-CD4 mAb Leu 3 and with the PE-labelled anti-CD8 mAb Leu (both from Becton Dickinson) in PBS supplemented with 0.1% bovine serum albumin (Sigma) and 0.01% NaN₃ for 30 min at 4 °C. After three washes, the cells were analysed on a FACScan (Becton Dickinson).

Fig. 2 Induction of CD8 messenger RNA in the T-cell clones CD-206 (lanes 1 and 2), CD-129 (lanes 3 and 4) and CD-30 (lanes 5 and 6). Cells were activated weekly for a period of three weeks in the presence of 20 U ml⁻¹ of IL-2 (lanes 1, 3 and 5) or 200 U ml⁻¹ IL-4 (lanes 2, 4 and 6). Six days after the last activation, the expression of CD8 (a), CD4 (b) and β -actin (c) genes was determined by Northern analysis. A CD4⁻CD8⁺ clone (lane 7) and a CD4⁺CD8⁻ clone (lane 8) were included as controls.

Methods. Total cytoplasmic RNA was isolated from 10⁶ cells by the method of Berger and Birkenmeier²³. Briefly, cells were washed once in PBS resuspended in 1 ml lysis buffer (10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 1.5 mM MgCl₂, 10 mM ribonucleoside vanadyl complexes (New England Biolabs) and Nonidet P₄₀ (NP-40) was added to a final concentration of 0.4%. Lysis occurred during incubation on ice for 5 min and nuclei were subsequently removed by centrifugation at 3,000 r.p.m. for 5 min. Supernatants were transferred to new tubes (Falcon 2063), 0.125 ml SDS mix (4% SDS, 0.8 M Tris-HCl pH 9.0, 50 mM EDTA) was added and proteins were removed by three phenol extractions with equal volumes of 0.1 M Tris-HCl, pH 8.0, saturated phenol (Gibco BRL). Total cytoplasmic RNA was collected by two ethanol precipitations, separated according to size on 1% agarose gels containing 6.6% formaldehyde and subsequently transferred to Nytran nylon membranes (Schleicher and Schuell) according to the manufacturer's instructions. The 1,737-base-pair (bp) *Bam*HI fragment of pcDNA-hu-CD4 (J. Dixon, unpublished results), the 1,200-bp *Xho*I fragment of CDM8-CD8 (B. Seed, unpublished results) and the 1,200-bp *Pst*I fragment of pAL (β -actin) (C. Goridis, unpublished results) were labelled to a specific activity of 5 $\times$ 10⁸ c.p.m. μ g⁻¹ and used as probes. Hybridizations were performed under stringent conditions as described²⁴ with final washes in 0.2 $\times$ SSC and 0.1% SDS at 65 $^{\circ}$ C.



T cells were stimulated with irradiated cells of the Epstein-Barr virus (EBV)-transformed B-cell line JY in the presence of the anti IL-2 monoclonal antibody (mAb) DMS-1 (ref. 5) or a rabbit anti-IL-4 antiserum⁶ for six days and then cloned by limiting dilution with irradiated peripheral blood leukocytes (PBL) (4,000 rad) and JY cells (5,000 rad) as feeder cells in the presence of either IL-4 plus DMS-1 or IL-2 plus the rabbit anti-IL-4 antibody. The cloning efficiency of the T cells with IL-4 as growth factor was 17% which was slightly higher than that with IL-2 (12%) (Table 1). Furthermore, 49% of the clones isolated in IL-4 were double positives (Table 1). In contrast, none of the clones isolated in IL-2 expressed CD4 and CD8 simultaneously. The bulk culture from which the IL-4 clones were obtained did not contain detectable double positive cells (results not shown). Thus, it is very unlikely that the CD4⁺CD8⁺ clones isolated in IL-4 originated from the undetectable quantities of CD4⁺CD8⁺ T cells that might have been present in the starting population. The stability of the expression of CD8 on these double positive T-cell clones was dependent on the pres-

ence of IL-4 in the culture: the expression of CD8 decreased when a representative sample from IL-4 isolated double positive clone CD-129 was activated and cultured in IL-2 (Fig. 1a). When clone CD-129 was maintained in IL-4, CD8 expression remained stable (Fig. 1b). To show that IL-4 can induce CD8 on cloned CD4⁺CD8⁻ cells, one such clone, CD-206, was activated and cultured in IL-4 for 21 days. A considerable proportion of CD-206 cells co-expressed CD4 and CD8 after culturing in IL-4 (Fig. 1c); after culturing in IL-2 the cells remained single positive for CD4 (Fig. 1d).

To confirm that the cell-surface expression of CD8 by IL-4 was due to *de novo* synthesis, the expression of CD8 messenger RNA (mRNA) was investigated. T-cell clone CD-206 (CD4⁺CD8⁻) that was isolated and maintained in IL-2 did not express CD8 mRNA (Fig. 2a, lane 1). CD8 mRNA was induced after weekly stimulation for a period of three weeks in the presence of IL-4 (Fig. 2a, lane 2). Culturing in IL-2 of the CD4⁺CD8⁺ clones CD-129 and CD-30, that were isolated in IL-4, resulted in a decrease in CD8 mRNA expression (Fig. 2a,

Table 1 Cloning efficiency and phenotype of T-cell clones obtained in IL-2 and in IL-4

Lymphokine added	No. of wells seeded	No. of growing clones	Cloning efficiency* (%)	CD4 ⁺ CD8 ⁻	No. of clones with phenotype†	CD4 ⁻ CD8 ⁺	CD4 ⁺ CD8 ⁺	CD4 ⁻ CD8 ⁻
IL-2	960	33	12	25	8	0	0	0
IL-4	960	47	17	4	19	23	1‡	1‡

PBL of a healthy donor (HLA A2, 24; B7, 60; Cw3, DR2, 13; DQ1) were stimulated with the irradiated (5,000 rad) EBV-transformed B-cell line JY (HLA A2, B7, DR4-6, DQw3) at a responder-to-stimulator cell ratio of 10:1 in the presence of either the anti-IL-2 mAb DMS-1 (ref. 5) or a rabbit anti-IL-4 antibody⁶. Six days later, those T cells stimulated with JY cells in the presence of anti-IL-4 were cloned in the presence of IL-2 and anti-IL-4; those stimulated with JY cells and anti-IL-2 were cloned in the presence of anti-IL-2 and IL-4. Cloning of the T cells was performed by limiting dilutions in 96-round-bottom-well plates (Flow Laboratories). A feeder-cell mixture was added with either the combination of 200 U ml⁻¹ IL-4 and a 1:100 dilution of DMS-1 ascites or of 20 U ml⁻¹ IL-2 and 50 μ g ml⁻¹ purified anti IL-4. Growing cultures were transferred between 10 and 18 days after the start of cloning to a 1-ml volume in a 24-well plate (Flow) and re-stimulated with feeder cells in the presence of 0.1 μ g ml⁻¹ phytohaemagglutinin (PHA) (Wellcome) and either recombinant IL-4 or IL-2. The clones were subsequently stimulated weekly with a feeder-cell mixture, PHA and the appropriate recombinant lymphokine. All cultures were in Yssel's medium¹⁸.

* Cloning efficiency was calculated according to the formula $-\ln[(\text{no. of negative wells}/\text{no. of total wells})/(\text{no. of cells per well})] \times 100$.

† The phenotype was determined as follows: the cells were incubated at 4 $^{\circ}$ C with mAbs for 30 min in phosphate buffered saline (PBS) plus 0.1% bovine serum albumin and 0.01% NaN₃ and after two washes these cells were labelled with F(ab')₂ fragments of goat anti-mouse (Grubb, Austria). The samples were analysed on a FACScan (Becton Dickinson). The mAbs used for phenotyping were SPV-T3b (anti-CD3)¹⁹, Leu 2 (anti-CD8) and Leu 3 (anti-CD4).

‡ This clone was CD3⁺CD4⁻CD8⁻ and reacted with the anti-T-cell δ -antibody TCR δ ¹ (ref. 20). No. = number.

Table 2 Effect of an anti-CD8 mAb on cytotoxicity of an IL-4-isolated clone

Lymphokine in which the clone was cultured	Target cell	Medium	OKT-4A anti-CD4	% Lysis in the presence of WT-81 anti-CD8	SPV-L14 anti-CD6
IL-4	JY	57	7	56	ND
	Daudi	0			
	Daudi + 10 ⁻⁴ anti-CD3	74	ND	43	67
	Daudi + 10 ⁻⁵ anti-CD3	25	ND	8	23
IL-2	JY	68	24	65	ND
	Daudi	0			
	Daudi + 10 ⁻⁴ anti-CD3	74	ND	70	76
	Daudi + 10 ⁻⁵ anti-CD3	37	ND	29	42

The CTL clone CD-505 was isolated in IL-4 and expressed CD4 and CD8 simultaneously. The clone was stimulated with feeder cells in the presence of either IL-2 or IL-4. The level of expression of CD8 on the in IL-2 cultured cells was only 20% of that expressed by the cells cultured in IL-4. Cytotoxic activity was determined with a standard ⁵¹Cr release assay as described previously¹⁰ in U-bottom microtitre plates (Nunc). The ratio of effector to target cell was 10:1. To test the effect of the mAbs SPV-L14²¹ (provided by Dr H. Yssel), OKT-4A (Ortho) and WT-81 (ref. 22), the effector cells were pre-incubated with 10 µg ml⁻¹ OKT-4A and a 1:600 dilutions of ascites of WT-81 or SPV-L14 for 20 min at room temperature. Daudi cells were pre-incubated with the indicated dilution of the anti-CD3 mAb SPV-T3b ascites fluid for 20 min before addition of the effector cells. ND, not done.

lanes 3 and 5), compared with the CD8 mRNA expression of these clones cultured in IL-4 (Fig. 2a, lanes 4 and 6). Furthermore, mRNA expression of CD4 (Fig. 2b) is not affected by culturing the clones in IL-4. These results indicate that IL-4 induces transcription of the CD8 gene, resulting in cell-surface expression of this antigen.

Blue and co-workers noted previously^{3,7} that factor(s) produced by activated T cells can maintain simultaneous expression of CD4 and CD8 on T cells, but they did not identify these factors. The results presented here indicate that IL-4 is likely to be the factor responsible. It is possible that in addition to IL-4, other factors (produced by the irradiated feeder cells which were present during stimulation of CD4⁺ cells in our study) might contribute to the induction of CD8.

Functional studies indicated that the CD4⁺CD8⁺ cytotoxic T-lymphocyte (CTL) clone CD-505 which was isolated in IL-4 recognizes HLA-DR, because the cytotoxicity of the CD4⁺CD8⁺ T-cell clone CD-505 isolated in IL-4 against JY was blocked by a monoclonal anti-HLA-DR antibody, but not by the anti-class I major histocompatibility complex (MHC) antibody W6-32 (results not shown). The class-II-specific CTL activity mediated by CD-505 was blocked by the anti-CD4 monoclonal antibody OKT-4A but not by the anti-CD8 mAb WT-81 (Table 2). These results are consistent with CD4, but not CD8 being involved in the antigen-specific cytotoxic activity of CTL with a T-cell antigen receptor (TCR) specific for class II MHC antigens⁸⁻¹⁰. However, WT-81 inhibits the anti-CD3-induced cytolytic activity mediated by CD-505 against the class I MHC-negative cell line, Daudi. In this situation, the specificity of the TCR is not relevant because this anti-CD3 mAb replaces the specific antigen^{11,12}. This blocking effect of WT-81 seemed to be specific for the anti-CD8 mAb as the anti-CD6 mAb SPV-L14, which is of the same immunoglobulin (Ig) G class as WT-81, did not affect the cytolytic activity induced by anti-CD3. Perhaps more importantly, WT-81 only marginally blocked the anti-CD3-induced cytotoxicity mediated by CD-505 cultured in IL-2, which expressed fivefold less CD8 (not shown) than the CD-505 cells cultured in IL-4. These results indicate that CD8 induced by IL-4 can be functional.

Recently, it has been shown that transfection of complementary DNA of a class I-MHC-specific TCR into a murine hybridoma transferred the specificity of the transfected TCR only if a CD8 cDNA clone was co-transfected¹³. This indicates that in certain situations, a class-I-specific TCR can only recognize its antigen if CD8 is present. Hence, in principle, the co-expression of CD8 on IL-4-cultured CD4⁺ T cells specific for certain peptides in the context of class II antigens may allow these cells to recognize different peptides presented by class I

antigens. This is not a remote possibility as human TCRs that are cross-reactive with class I and class II MHC antigens have been described¹⁴. Because the induced expression of CD8 is dependent on the continuous presence of IL-4 and may therefore be transient, this recognition of peptides in the context of class I MHC by CD4⁺CD8⁺ T cells would be self-limiting. The physiological significance of the ability of CD4⁺ T cells to temporarily acquire an extra specificity is unknown, but it may be important in interactions between T cells. Interestingly, double positive T-cell clones have been isolated from patients suffering lepromatous lepra¹⁵. These clones were shown to have suppressor cell activity. Elevated numbers of double positive T cells have been observed in the joint fluid of patients suffering from juvenile rheumatoid arthritis¹⁶ and in PBL of myasthenia gravis patients¹⁷. On the basis of our results, a possible role of IL-4 in the appearance of elevated numbers of double positive cells in these autoimmune disorders can be envisaged.

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Rings of negatively charged amino acids determine the acetylcholine receptor channel conductance

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The structure-function relationship of the nicotinic acetylcholine receptor (AChR) has been effectively studied by the combination of complementary DNA manipulation and single-channel current analysis¹⁻⁶. Previous work with chimaeras between the *Torpedo californica* and bovine AChR δ -subunits has shown that the region comprising the hydrophobic segment M2 and its vicinity contains an important determinant of the rate of ion transport through the AChR channel⁵. It has also been suggested that this region is responsible for the reduction in channel conductance caused by divalent cations⁵ and that segment M2 contributes to the binding site of noncompetitive antagonists^{7,8}. To identify those amino acid residues that interact with permeating ions, we have introduced various point mutations into the *Torpedo* AChR subunit cDNAs to alter the net charge of the charged or glutamine residues around the proposed transmembrane segments⁹⁻¹⁵. The single-channel conductance properties of these AChR mutants expressed in *Xenopus*

laevis oocytes indicate that three clusters of negatively charged and glutamine residues neighbouring segment M2 of the α -, β -, γ - and δ -subunits, probably forming three anionic rings, are major determinants of the rate of ion transport.

The regions of the *T. californica* AChR subunits lying between segments M1 and M2 and between segments M2 and M3 (referred to as the M1-M2 portion and the M2-M3 portion, respectively) contain four clusters of negatively charged and polar amino acid residues (Fig. 1). These are located at the α -subunit positions α D238, α E241, α E262 and α S266 (amino acid residues followed by residue numbers⁹⁻¹¹) and the equivalent positions of the three other subunits. The single-channel current-voltage (*i*-*V*) relation of acetylcholine (ACh)-activated currents (obtained in a nominally divalent-cation-free, symmetrical K⁺-rich solution) of the wild-type AChR channel (Fig. 2a) is compared with those of the AChR channels with the α -subunit mutation α E262K (Fig. 2b) or α D238K (Fig. 2c) (amino acid residues in the wild-type and the mutant preceding and following the number of the altered residue, respectively). Both the mutations result in decreased conductance for both inward and outward currents. The *i*-*V* relation of the wild-type channel is nearly symmetrical, whereas those of the mutant channels are asymmetrical, indicating outward (α E262K) or inward (α D238K) rectification of ion transport.

Conductance properties were studied for other AChR mutants in which the residues at the α -subunit position α E262 and at the equivalent positions of the three other subunits were systematically altered. In Fig. 2d, the conductances for inward currents of these mutants and of various combinations of them are plotted as a function of the decrease in the total number of net negative charges caused by the mutations. An approximately inverse relationship is found between channel conductance and change in total net negative charge. The data on the α -subunit mutations also indicate that the charge of the side chains is a more important determinant of the rate of ion transport than their size. These results, together with the pseudosymmetrical

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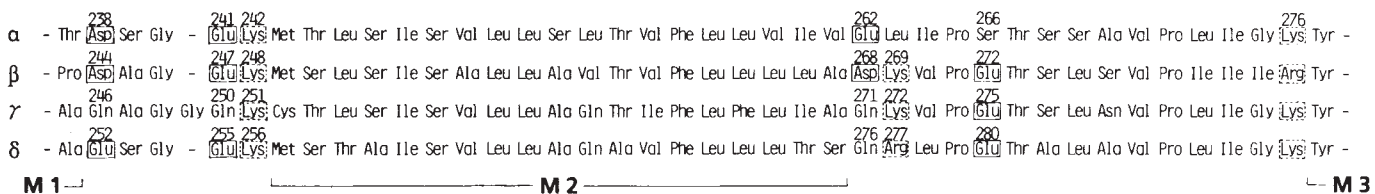


Fig. 1 Regions surrounding segment M2 of the α -, β -, γ - and δ -subunits of the *T. californica* AChR subjected to point mutations. The relevant amino acid sequences⁹⁻¹¹ are aligned^{11,26}. The numbers⁹⁻¹¹ of the relevant amino acid residues and the positions of the hydrophobic segments M1, M2 and M3 (defined in ref. 11) are indicated. Negatively charged residues are boxed with solid lines, and positively charged residues with broken lines.

Methods. cDNAs encoding *T. californica* AChR α -, β -, γ - and δ -subunit mutants were constructed^{2,6,27,28} using synthetic oligodeoxyribonucleotides prepared with an automatic DNA synthesizer (Applied Biosystems); some silent mutations were also introduced by this strategy. The plasmids constructed differ from the parental plasmids (given in parentheses) as follows (the substituted nucleotides with residue numbers⁹⁻¹¹ are given, and the plasmids carrying mutated cDNAs linked with the SP6 promoter²⁹ are named after the mutant specification). pSP α 6 (pSP α , ref. 2): A 573, 597, 687; T 594, 648; G627; C630, 651, 690, 691; AGC 676-678. pSP α 7 (pSP α): C 717, 748, 750; G 720, 732, 762; A 735, 741, 744, 747; TCG 736-738; T 751; AAGCT 753-757; the junctional sequence between the 3' end of the α -subunit cDNA and the *Eco*RI site of the vector taken from pSP α (ref. 2, referred to as junctional sequence). pSP α R209E (pSP α 6): G 625; A 626. pSP α R209Q (pSP α 6): A 626. pSP α D238R (pSP α 6): C 712; G 713. pSP α D238K (pSP α): T 648; C 651, 690, 691; AGC 676-678; A 687, 712; G 714. pSP α D238N (pSP α 7): T 648; C 651, 690, 691, 703; AGC 676-678; A 687, 712; G 693, 705. pSP α D238E (pSP α 6): G 714. pSP α E241Q (pSP α 7): C 721. pSP α E241D (pSP α 7): T 720, 723, 732. pSP α K242E (pSP α): T 648, 751, 757; C 651, 690, 691, 748, 750, 756; AGC 676-678; A 687, 726, 735, 741, 744, 747, 753, 754; G 724, 755, 762; TCG 736-738. pSP α K242Q (pSP α 7): T 720, 732; C 724; A 726. pSP α E262R (pSP α 6): C 784; G 785. pSP α E262K (pSP α): A 784; junctional sequence. pSP α E262Q (pSP α): C 784; junctional sequence. pSP α E262D (pSP α 6): C 786. pSP α K276E (pSP α 8, see below): G 826. pSP α R301E (pSP α 8): GAA 901-903. pSP α D371K (pSP α 4, ref. 6): G 1065, 1113; C 1071; A 1111. pSP α E377K (pSP α 4): A 1129. pSP α E384K (pSP α 4): A 1150. pSP α D389K (pSP α 4): A 1162, 1165, 1167; G 1163. pSP α E390K (pSP α 4): A 1162, 1168; G 1163. pSP α E391K (pSP α 4): A 1162, 1171; G 1163. pSP α (E397K-E398K) (pSP α 5, ref. 6): A 1189, 1192; G 1194. pSP α D407K (pSP α 5): A 1219, 1221; C 1266. pSP α D407N (pSP α 5): A 1219; C 1221, 1266. pSP α E432K (pSP α 4): A1294; G 1296. pSP β D244K (pSP β , ref. 2): A 730; G732. pSP β E247Q (pSP β): C 739. pSP β K248E (pSP β): G 742. pSP β D268K (pSP β): A 802; G 804. pSP β K269E (pSP β): G 805. pSP β E272K (pSP β): A 814. pSP γ Q246K (pSP γ , ref. 2): A 736. pSP γ Q250K (pSP γ): A 748. pSP γ K251E (pSP γ): G 751. pSP γ Q271K (pSP γ): A 811. pSP γ K272E (pSP γ): G814. pSP γ E275K (pSP γ): A 823. pSP δ E252K (pSP δ , ref. 2): A 754. pSP δ E255Q (pSP δ): C 763. pSP δ K256E (pSP δ): G 766. pSP δ Q276K (pSP δ): A 826. pSP δ R277E (pSP δ): G 829; A 830. pSP δ E280K (pSP δ): A 838. The plasmid pSP α 8 was constructed by ligating the 2.1-kilobase-pair (kb) *Hind*III fragment from pSP α 6 and the 3.0-kb *Sac*I/*Hind*III fragment from pSP α after they had been blunt-ended. Messenger RNAs specific for mutant and wild-type AChR subunits were synthesized *in vitro*^{2,5,6}, using *Eco*RI- or *Sma*I-cleaved plasmids carrying the respective cDNAs as templates.

Fig. 2 Effects of point mutations in the M2-M3 and M1-M2 portions. *a-c*, Single-channel *i-V* relations of the wild-type AChR channel (*a*) and the mutant channels with α E262K (*b*) or α D238K (*c*). Each symbol represents a mean of at least four amplitude measurements within 4-mV intervals. Continuous lines represent fitted 5th-order polynomials. *d, e*, Changes in single-channel conductance with decreases in the total number of net negative charges. The chord conductances at -100 mV membrane potential of the AChR channels with a mutation or mutations at α E262 and/or the equivalent positions (*d*) or the chord conductances at $+100$ mV membrane potential of the AChR channels with a mutation or mutations at α D238 and/or the equivalent positions (*e*) are plotted against the reduction in the total number of net negative charges. A change from a negatively-charged to a positively-charged residue is scored as two and a change from a negatively-charged to a neutral residue or from a neutral to a positively charged residue as one. Scores are doubled for mutations in the α -subunit because of the $\alpha_2\beta\gamma\delta$ stoichiometry and are summed in case of multiple mutations. Each symbol, followed by the mutant specification, is the mean of 3-5 experiments, the standard deviation being at most 3 pS. WT refers to the wild-type AChR channel.

Methods. *X. laevis* oocytes were injected² with the four kinds of mutant or wild-type mRNAs (α -, β -, γ - and δ -subunit-specific mRNAs in a molar ratio of 2:1:1:1) and were incubated¹ for 3-5 days. Single-channel current measurements were made at $12 \pm 1^\circ\text{C}$ in inside-out membrane patches isolated from the oocytes³⁰. The bath solution contained 100 mM KCl, 10 mM EGTA and 10 mM HEPES; the solution was adjusted to pH 7.2 with KOH. The pipette solution was the same as the bath solution, except that ACh was added in a final concentration of 0.5-4 μM . The *i-V* relations were obtained⁵ in the voltage range between -200 mV and $+200$ mV. In some experiments, the *i-V* relations were shifted by a few millivolts so that the zero current potential was 0 mV.

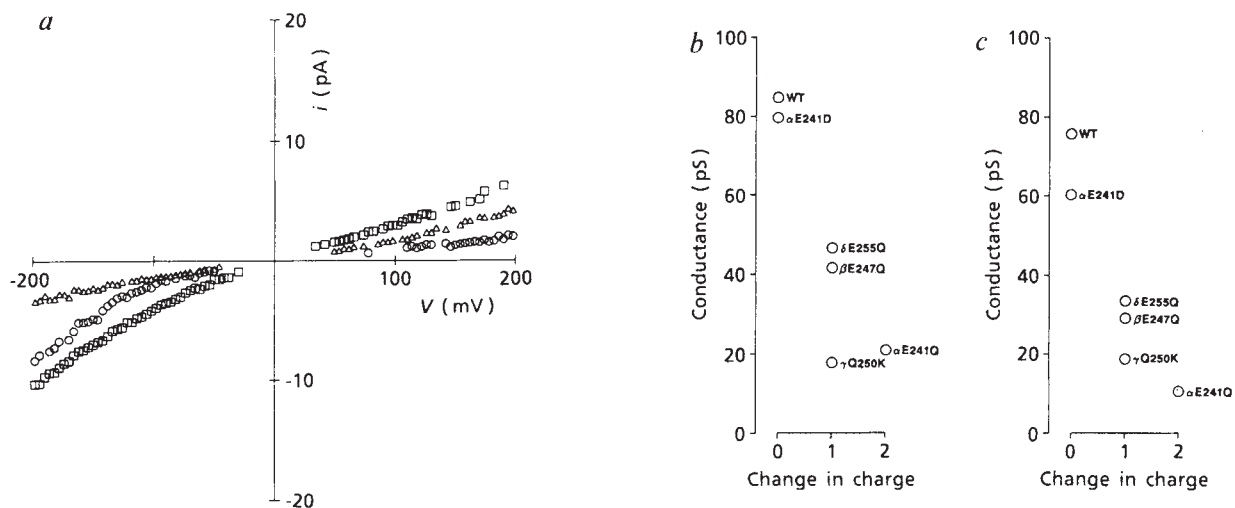
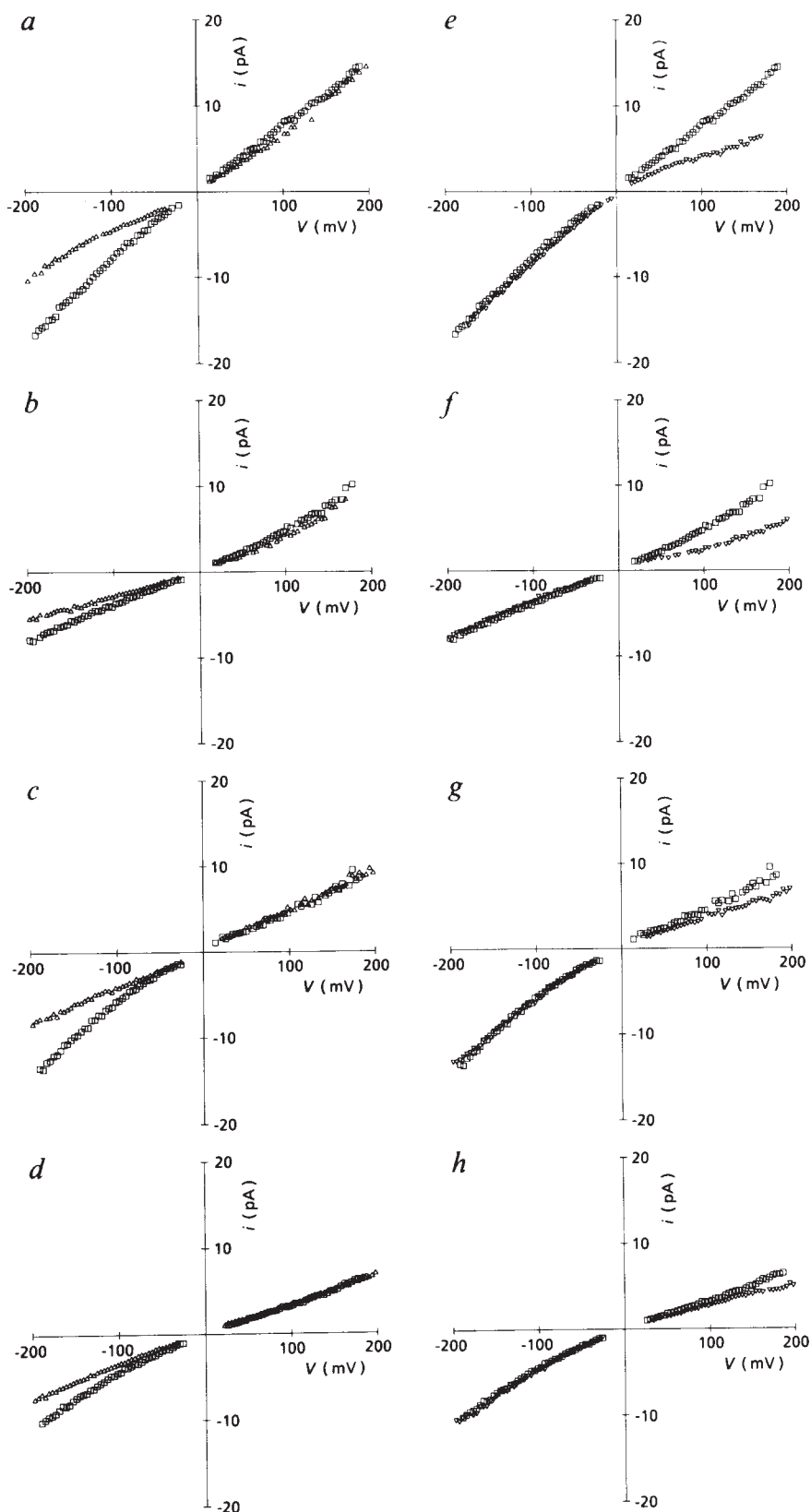


Fig. 3 Single-channel conductances of the AChR channels with mutations in the intermediate ring. *a*, *i-V* relations of the AChR channels with a mutation in the α - (α E241Q, circles), β - (β E247Q, squares) or γ -subunit (γ Q250K, triangles); the *i-V* relation of the AChR channel with a mutation in the δ -subunit (δ E255Q) is shown in Fig. 4d, h (squares). *b, c*, Changes in single-channel conductance with decreases in the total number of net negative charges. The chord conductances at membrane potentials of -100 mV (*b*) and $+100$ mV (*c*) are plotted as in Fig. 2d, e. The experimental conditions were as described in Fig. 2 legend.

Fig. 4 Sidedness of Mg^{2+} effects on the i - V relations of the wild-type AChR channel (*a, e*) and the mutant channels with α E262K (*b, f*), α D238K (*c, g*) or δ E255Q (*d, h*). In each panel, two i - V relations are shown. Square symbols indicate control experiments under the conditions described in Fig. 2 legend. Triangular symbols indicate experiments under asymmetrical conditions with 0.5 mM Mg^{2+} either on the extracellular side (*a-d*) or on the intracellular side (*e-h*). The Mg^{2+} -containing solution was composed of 100 mM KCl, 0.5 mM $MgCl_2$ and 10 mM HEPES; the solution was adjusted to pH 7.2 by adding KOH. The nominally divalent-cation-free solution specified in Fig. 2 legend was used for the other side. ACh was added to the pipette solution. For further experimental details, see Fig. 2 legend. The effects of extracellular and intracellular Mg^{2+} on mean chord conductances (3–5 experiments) at membrane potentials of -150 mV and $+150$ mV, respectively, are expressed as percentage decreases relative to mean control values (3–7 experiments) without Mg^{2+} . The values for inward and outward currents respectively are 42% and 50% for the wild-type; 29% and 48% for α E262K; 43% and 17% for α D238K; and 31% and 18% for δ E255Q. The standard deviation for each conductance measurement was at most 4 pS.



orientation of the AChR subunits assembled in a molar stoichiometry of $\alpha_2\beta\gamma\delta$ (ref. 16), indicate that the negatively charged and glutamine residues in this cluster are nearly equidistant from the channel axis, forming a ring structure. Analogous experiments on AChR channels mutagenized in the cluster at α D238 and the equivalent positions reveal a similar inverse relationship between channel conductance for outward current

and change in total net negative charge, indicating that there is another ring of negatively-charged and glutamine residues (Fig. 2e). Mutations in the two anionic rings evoke opposite directions of rectification of the i - V relation (exemplified in Fig. 2b, c), implying that the rings are on opposite sides of the membrane.

Figure 3a–c shows the effects of mutating residues in the cluster at α E241 and the equivalent positions. Reducing the net

negative charge of the individual residues in this intermediate cluster has a much stronger effect (Fig. 3b, c) than when the residues of the corresponding subunits in the two other clusters are mutated (Fig. 2d, e). The intermediate cluster of glutamic acid and glutamine residues, probably also forming a ring-like structure, is therefore more important in determining the rate of ion transport than the two other anionic rings. Notably, the γ -subunit mutation γ Q250K causes a greater reduction in conductance than would be expected from the decrease in net negative charge resulting from the mutation. Furthermore, the AChR channel with the γ -subunit mutation shows virtually no rectification of the i - V relation, whereas inward rectification is evoked by the α - (α E241Q and α E241D), β - (β E247Q) or δ -subunit mutation (δ E255Q) (Fig. 3a-c; see also Fig. 4d, h). It is possible that the individual residues of the intermediate ring do not occupy strictly equivalent positions in the electrical profile of the channel. The glutamic acid residues in the α -, β - and δ -subunits may be located closer to the anionic ring at α D238 than the glutamine residue in the γ -subunit, which may be closer to the centre of the electrical profile. In this context, it could be relevant that the γ -subunit has an insertion of a glycine in the M1-M2 portion compared with the other subunits (Fig. 1). The difference in rectification and the variable extents of reduction in conductance may indicate that, in the intermediate ring, not only the net negative charges of the constituent residues but also other factors, such as their size, position and interaction, are involved in determining the rate of ion transport. We conclude that the intermediate ring is located between the two other rings, probably nearer to the anionic ring at α D238, and is close to the narrowest part of the channel.

When 0.5 mM Mg^{2+} is added to the extracellular side (Fig. 4a-d), the inward current of the mutant channel with α E262K (Fig. 4b) is found to be less sensitive to Mg^{2+} than that of the wild-type channel (Fig. 4a). In contrast, the mutant channel with α D238K (Fig. 4c) shows nearly the same relative decrease in conductance for inward current as the wild-type. There is no significant change in the outward current of either channel. When 0.5 mM Mg^{2+} is present on the intracellular side (Fig. 4e-h), the outward current of the channel with α D238K (Fig. 4g) is less affected than that of the wild-type channel (Fig. 4e). In contrast, the relative decrease in conductance for outward current is similar in the channel with α E262K (Fig. 4f) and in the wild-type channel. The inward current is essentially unaffected in the wild-type and mutant channels. These results indicate that the anionic ring at α E262 and that at α D238 are involved in interactions with Mg^{2+} , suggesting that these rings constitute part of the extracellular and the cytoplasmic portion of the channel, respectively. Figure 4d, h shows that the inward and the outward current of the channel with δ E255Q are less sensitive to extracellular and intracellular Mg^{2+} , respectively, than the currents of the wild-type channel. The intermediate ring is therefore involved in the interaction with Mg^{2+} . The reduced sensitivity to Mg^{2+} on both sides provides further evidence that this ring is located between the extracellular and cytoplasmic rings.

No significant changes in channel conductance result from the following mutations (data not shown): β K248E, γ K251E, δ K256E, β K269E, γ K272E, δ R277E, β E272K, γ E275K, δ E280K, β E272K- γ E275K- δ E280K, α K276E, α R301E and α E432K (neighbouring segment M2, M3 or M4); α D371K, α E377K, α E384K, α D389K, α E390K, α E391K and α (E397K-E398K) in the amphipathic segment MA (refs 14, 15) and the neighbouring regions. α D407N (neighbouring segment M4) causes a slight decrease in conductance. The data on segment MA are consistent with our previous studies, suggesting that this segment is dispensable for ACh responsiveness, although it is important for efficient expression of AChR in the plasma membrane⁶. Mutations of some charged residues (α R209E, α R209Q, α K242E, α K242Q and α D407K) cause a failure to observe channel openings, which is probably due to

insufficient functional expression of AChR.

We have identified three clusters of negatively charged and glutamine residues neighbouring the hydrophobic segment M2 as major determinants of the AChR channel conductance for monovalent cations and as sites of interaction with divalent cations. Our results suggest that these amino acid residues form three anionic rings. The membrane topology of the three rings is based largely on the sidedness of Mg^{2+} effects, which provides evidence that the hydrophobic segment M2 is a transmembrane segment with the M2-M3 portion on the extracellular side and with the M1-M2 portion on the cytoplasmic side. Thus, the extracellular ring is located near the external mouth and the cytoplasmic ring near the internal mouth of the channel. The intermediate ring is positioned between the two other rings and may form a narrow channel constriction. The location of both the intermediate and the cytoplasmic ring in the M1-M2 portion suggests that this portion actually forms part of the transmembrane segment containing segment M2. This transmembrane segment probably constitutes at least part of the channel lining. However, our results do not exclude the possibility that other transmembrane segments also take part in forming the channel lining.

In the regions neighbouring segment M2, the mammalian muscle AChR ϵ -subunit has two more net negative charges than the γ -subunit, one in each of the extracellular and cytoplasmic rings¹⁷⁻¹⁹. This may account for the higher channel conductance of the AChR composed of the α -, β -, δ - and ϵ -subunits compared with the AChR composed of the α -, β -, γ - and δ -subunits^{4,19}. Interestingly, the *T. californica* electroplax AChR γ -subunit is equivalent to the ϵ - rather than to the γ -subunit of the mammalian muscle AChR with respect to the amino acid residues in the three anionic rings^{11,12,17-22}. Our conclusion that rings containing charged amino acid residues are major determinants of the rate of ion transport may also be valid for other multisubunit ligand-gated channels, such as the neuronal AChR²³, the GABA_A receptor²⁴ and the glycine receptor²⁵.

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Defective co-translational formation of disulphide bonds in protein disulphide-isomerase-deficient microsomes

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The formation of disulphide bonds in mammalian secretory and cell-surface proteins occurs in the lumen of the endoplasmic reticulum and is believed to be catalysed by the enzyme protein disulphide-isomerase (PDI)¹. The evidence for this physiological role for PDI is circumstantial and relates to the cell and tissue distribution of the enzyme, its developmental behaviour and its catalytic properties *in vitro*²⁻⁴. A clear requirement for PDI in the correct folding or assembly of disulphide-bonded proteins during biosynthesis has not been demonstrated. We have prepared dog pancreas microsomes which are deficient in soluble luminal proteins, including PDI, but which are still able to translocate and process proteins synthesized *in vitro*^{5,6}. Using the formation of intramolecular disulphide bonds during the *in vitro* synthesis of γ -gliadin⁷, a wheat storage protein, as a model, we have demonstrated that these microsomes are defective in co-translational formation of disulphide bonds. Reconstitution of these microsomes with purified PDI reverses this defect.

A model system was used to determine the requirements for disulphide-bond formation in protein biosynthesis. This included a conventional rabbit reticulocyte lysate⁸ translation system which had been optimized in preliminary experiments for the formation of disulphide bonds during translation^{7,9,10}. The messenger RNA used codes for a modified γ -gliadin and was obtained by *in vitro* transcription of plasmid pUKC1001 (see legend to Fig. 1). The corresponding protein contains eight cysteine residues and is believed to form four intramolecular disulphide bonds¹¹. Translation was carried out in the presence of three preparations of dog pancreas microsomes. These were: (1) conventional 'native' microsomes; (2) pH 9-washed microsomes subjected to a treatment that removes soluble luminal proteins but yields intact microsomes capable of translocating and processing proteins synthesized *in vitro*^{5,6}; and (3) a preparation obtained by specifically reconstituting pH 9-washed microsomes with purified bovine PDI. The assay system used for monitoring formation of disulphide bonds was SDS-PAGE analysis of translation products that were separated both under conventional reducing conditions and in conditions where pre-existing disulphide bonds are preserved; many intramolecular disulphide-bonded proteins show greater electrophoretic mobility under the latter conditions¹². A full account of the translation and assay systems for co-translational translocation and disulphide formation is given elsewhere⁷.

To investigate the process of co-translational disulphide formation, translation was carried out in the presence of native untreated dog pancreas microsomes or in the presence of pH 9-washed microsomes. At various times after initiation of translation, translation products were analysed by SDS-PAGE in non-reducing conditions to monitor the thiol/disulphide redox status of the translation products. Figure 1a shows that for translation in the presence of native microsomes most of the translation product appears as the faster migrating band, and is therefore disulphide-bonded within 15 min of the start of translation. In contrast, for translation carried out in presence of pH 9-washed microsomes, electrophoresis shows that the faster-migrating band (corresponding to disulphide-bonded translation product) appears slowly over a period of 30 min (Fig. 1b) and there is a prominent, more slowly-migrating band throughout, corresponding to reduced translation product. Comparison of SDS-PAGE profiles of control microsomes,

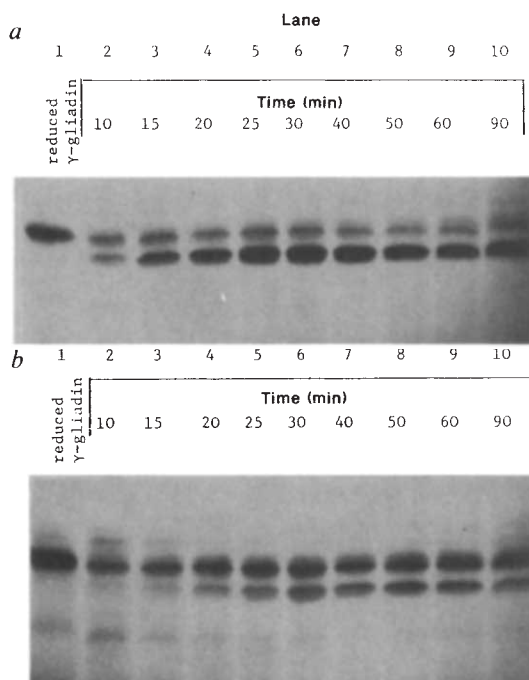


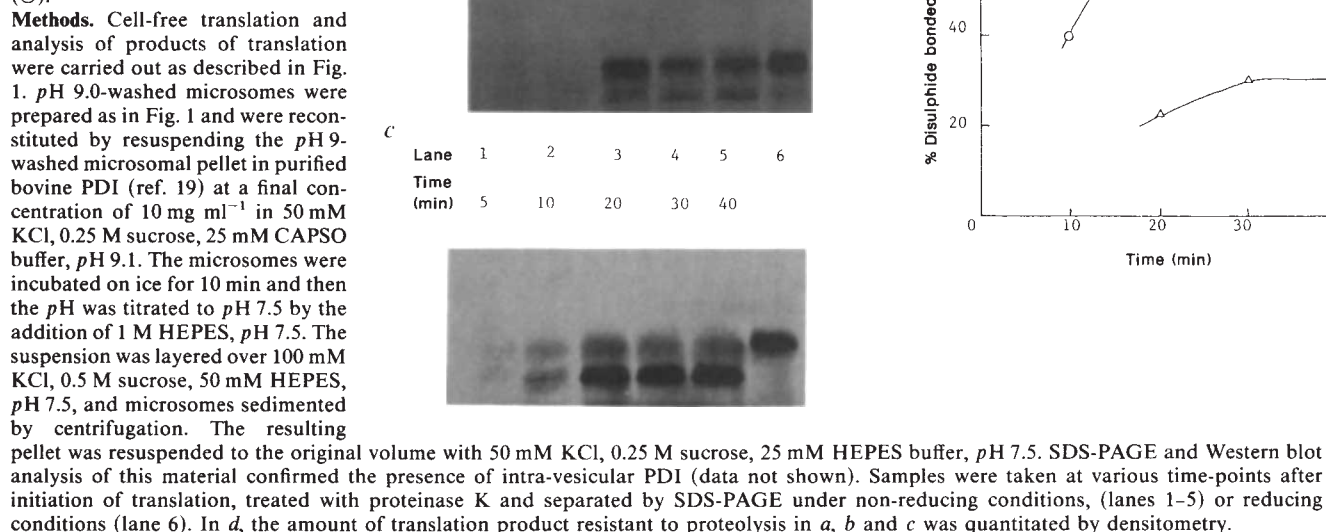
Fig. 1 Time course of disulphide-bond formation in newly synthesized protein in the presence of native or pH 9-washed microsomal vesicles.

Methods. Synthesis of γ -gliadin mRNA from pUKC1001 using T₇ RNA polymerase and synthesis of [³⁵S]methionine-labelled γ -gliadin in a reticulocyte cell-free system in the presence of dog pancreatic microsomal vesicles have been described⁷. Sequencing of this clone showed that it differed from the published sequence¹¹ in lacking 159 base pairs, giving an in-frame deletion of 53 residues in the proline- and glutamine-rich repeat region, which does not contain cysteine and forms a distinct domain from the disulphide-bonded C-terminal portion of the molecule. Rabbit reticulocyte lysate was prepared by the method of Pelham and Jackson⁸ without supplementing with DTT. pH 9-washed microsomes were prepared by adding 1.3 ml 50 mM KCl, 0.25 M sucrose, 25 mM CAPSO buffer, pH 9.1, to 200 μ l dog pancreatic microsomes (17 mg ml⁻¹), hand-homogenizing the suspension, layering it over 1 ml 100 mM KCl, 0.5 M sucrose, 50 mM HEPES, pH 7.5 and centrifuging at 109,000g for 90 min at 4°C. The resulting pellet was resuspended to a final volume of 200 μ l with 50 mM KCl, 0.25 M sucrose, 25 mM HEPES buffer, pH 7.5. Cell-free translation was carried out as described⁷ either in the presence of: a, native; or b, 'pH 9-washed' microsomes. Samples were taken at various time-points after initiation of translation, and separated through an 11% polyacrylamide gel¹⁸, under non-reducing conditions (lanes 2-10) as described⁷. Products of translation were also separated by SDS-PAGE under reducing conditions (lane 1). Gels were soaked in 10% (v/v) acetic acid, 10% (v/v) methanol for 30 min, in Amplify (Amersham) fluorographic reagent for 15 min, dried and exposed to pre-flashed Kodak XAR-5 autoradiographic film for 16 h.

pH 9-washed microsomes and the pH 9 extract shows that a band of relative molecular mass (M_r) 57,000 (57K) is almost completely released into the extract^{5,6}. Western blotting confirms that this is protein disulphide-isomerase and enzyme assay demonstrates that pH 9-washed microsomes retain less than 3% of control PDI activity, whereas 86% of the initial activity is recovered in the pH 9 extract^{5,6}.

To test the hypothesis that the defect shown in Fig. 1b was due to removal of PDI, we carried out a similar time-course experiment with native microsomes, pH 9-washed microsomes, and with pH 9-washed microsomes that had been specifically reconstituted with PDI (Fig. 2a, b, c). For accurate quantitation of the amounts of reduced and oxidized protein formed, the translation mixes were treated with proteinase K before SDS-

Fig. 2 Quantitation of disulphide-bond formation in newly synthesized protein in the presence of: *a*, native microsomes; *b*, pH-9 washed microsomes; and *c*, pH-9 washed microsomes reconstituted with purified PDI. *d*, Densitometry of translation product bands, which is plotted as a function of time from *a*, *b* and *c* gives the % of total protease-resistant translation product appearing in the disulphide-bonded form for native microsomes ($\blacktriangle$), pH 9-washed microsomes ($\triangle$) and pH-9 washed microsomes reconstituted with PDI ($\circ$).



PAGE analysis to eliminate translation products that had not been translocated into the microsome interior (compare Figs 1a and 2a). The bands were quantitated by densitometry and the proportion of disulphide-bonded product is plotted as a function of time in Fig. 2d. In native microsomes the translocated product is >70% disulphide-bonded at the earliest time-point and >90% disulphide-bonded from 20 min onwards, but in the pH 9-washed microsomes the disulphide-bonded product accumulates more slowly and is never more than 30% of the total. In the pH 9-washed microsomes reconstituted with PDI, the translation product is >40% disulphide-bonded at earlier time-points and >70% disulphide-bonded from 20 min onwards. In these experiments there was no difference in the level of proteinase K-resistant protein synthesis for translation in the presence of either native or treated microsomes (data not shown). Hence the pH 9-washed microsomes show a specific defect in the formation of disulphide bonds in newly synthesized and translocated protein which can be reversed by reconstituting the microsomes with purified PDI.

Previous work has shown that the co-translational formation of disulphide bonded proteins *in vitro* can be promoted by the addition of oxidizing agents such as diglutathione disulphide (GSSG)^{9,10}. In those studies, dithiothreitol (DTT) was added during the preparation of the rabbit reticulocyte lysate to increase the stability of the lysate preparation⁸. GSSG was then added to the lysate before initiation of translation to obtain a lysate that was competent in forming disulphide bonds in newly synthesized proteins. The lysate we used here was not supplemented with DTT and did not require the addition of GSSG for disulphide bonds to be formed during protein synthesis. We therefore prepared a lysate supplemented with DTT to investigate whether the deficiency in the formation of disulphide-bonded protein by pH 9-washed microsomes could be reversed by the addition of GSSG. In the presence of native microsomes

no disulphide-bonded product was seen in the absence of added GSSG (Fig. 3). At 1 mM GSSG a faster-migrating band appeared which was consistent with the formation of disulphide-bonded proteins. At higher concentrations the relative amount of disulphide-bonded protein increased but translation was inhibited. In the presence of pH 9-washed microsomes, no disulphide-bonded proteins were observed at any GSSG concentration. Inhibition of translation was more marked with these microsomes. Interestingly, more protein was synthesized at 1 mM GSSG than in the absence of added oxidant. These results clearly demonstrate that the deficiency of pH 9-washed microsomes in co-translational disulphide bond formation cannot be reversed

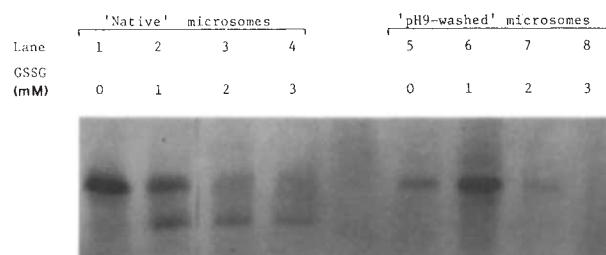


Fig. 3 Cell-free translation in DTT-supplemented lysate in presence of GSSG.

Methods. A rabbit reticulocyte lysate was prepared⁸ and supplemented with 1 mM DTT. Translation of γ -gliadin mRNA was in the presence of either native microsomes (lanes 1–4) or pH 9-washed microsomes (lanes 5–8). Increasing concentrations of GSSG were added before initiation of translation as indicated. Translation was for 15 min and translation products were treated with proteinase K and separated under non-reducing conditions as in Fig. 1.

by the presence of low relative molecular mass thiols and disulphides.

The results shown in Fig. 2 strongly imply that the defective co-translational formation of disulphide bonds observed in translations carried out in presence of pH 9-washed microsomes derives mainly from the loss of PDI. The extract from washing of microsomes at pH 9 contains other prominent proteins in addition to PDI with molecular masses of 94K and 78K; Western blotting confirms that BiP (grp 78) is preferentially extracted (data not shown). Thus it is likely that all the major luminal content proteins (reticulo endoplasmic¹³) are removed by pH 9 treatment, and more than one of these proteins may have roles in co-translational protein folding and disulphide formation. Much evidence is now accumulating that points to a function for BiP in the assembly of oligomeric secreted proteins¹⁴⁻¹⁶, but no folding activity has been demonstrated *in vitro*. On the other hand, PDI catalyses a range of thiol-disulphide interchange reactions *in vitro*, including the formation of native intramolecular and intermolecular disulphide bonds from denatured reduced proteins^{2,3} at rates consistent with the *in vivo*⁴ process. Furthermore, *in vivo* cross-linking has demonstrated direct association between PDI and nascent immunoglobulin chains¹⁷. We infer that it is primarily the loss of PDI from pH 9-washed membranes that causes the defect in co-translational disulphide formation (Figs 1b and 2b), and this

is confirmed in our reconstitution studies (Fig. 2c and d). These studies imply a direct role for PDI in the formation of disulphide bonds in protein biosynthesis.

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The role of protein surface charges in ion binding

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Protein engineering is a means of probing the role of electrostatic interactions in protein functions; this elegant technique has been applied to the elucidation of electrostatic effects in enzyme catalysis¹. Here we show how the use of mutant proteins allows the determination of the contributions of individual charges to the free energy of ion binding to proteins. We have investigated the importance of three negatively charged side chains in the binding of Ca^{2+} to bovine calbindin $\text{D}_{9\text{K}}$ (ref. 2): these are clustered around the calcium sites but are not directly involved as ligands. Each of these charges is found to contribute $\sim 7 \text{ kJ mol}^{-1}$ to the free energy of binding of two Ca^{2+} ions and to affect the cooperativity of Ca^{2+} binding. The influence of surface charges on ion binding to proteins may be more common than generally supposed and could have important consequences for protein function.

Calbindin $\text{D}_{9\text{K}}$ is an intracellular Ca^{2+} -binding protein with a structure that resembles the globular domains of the related proteins calmodulin, troponin C and parvalbumin. An important feature of these highly homologous proteins is the EF-hand³ Ca^{2+} -binding site characterized by its helix-loop-helix configuration. The pairwise arrangement of such sites permits cooperative Ca^{2+} -binding.

The presence in calbindin $\text{D}_{9\text{K}}$ of only two Ca^{2+} sites facilitates the interpretation of Ca^{2+} -binding studies and makes calbindin $\text{D}_{9\text{K}}$ an ideal system for exploring the molecular basis of the interactions within a pair of EF-hands.

The X-ray structure of bovine calbindin $\text{D}_{9\text{K}}$ (ref. 4) reveals a cluster of negatively charged amino-acid side chains on the protein surface around the Ca^{2+} sites. In this study we focus our attention on three of these residues—Glu 17, Asp 19 and

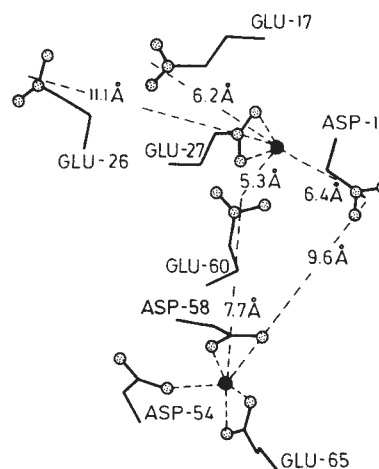


Fig. 1 The cluster of negative surface residues around the two sites of calbindin $\text{D}_{9\text{K}}$. According to X-ray studies⁴, only Glu-27, Asp-54, Asp-58 and Glu-65 are part of the inner ligand spheres of sites I and II respectively. Distances between the Ca^{2+} ions (●) and the non-ligated carboxylate groups (midpoint between oxygens) are indicated.

Glu 26—in the N-terminal Ca^{2+} -binding site (site I). The disposition in the X-ray structure of the carboxylate groups and the relevant inter-residue distances are schematically shown in Fig. 1. The contributions of these three charges to the free energy of binding of two Ca^{2+} ions and to the cooperativity of the Ca^{2+} binding have been assessed through a study of seven mutant proteins. The charges have been neutralized with a minimal change in side-chain structure and volume by substituting Asn for Asp and Gln for Glu. All possible single, double and triple mutants have been constructed (see Table 1). The proteins have been produced in *Escherichia coli* using synthetic genes⁵ and purified to homogeneity as shown by SDS-polyacrylamide and agarose gel electrophoresis, isoelectric focusing and ^1H NMR.

The macroscopic Ca^{2+} -binding constants, K_1 and K_2 , of the wild-type (M0) and mutant calbindins $\text{D}_{9\text{K}}$ are summarized in Table 1. The effects of the neutralization of the negative surface residues on the Ca^{2+} affinity of calbindin are striking. Compared

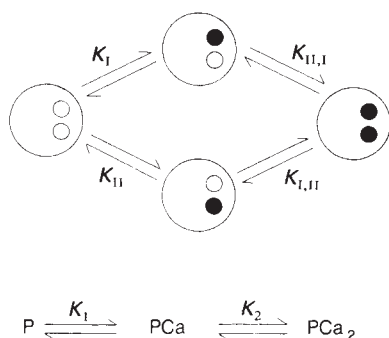


Fig. 2 Definition of microscopic (or site-) binding constants, K_I , K_{II} , $K_{I,II}$ and $K_{II,I}$ and macroscopic (or stoichiometric) binding constants, K_1 and K_2 . P represents the Ca^{2+} -free form of the protein, P·Ca a protein molecule with one Ca^{2+} ion bound (to either site I or site II) and P·Ca₂ a protein molecule with both sites occupied.

with M0, the Ca^{2+} affinity (both K_1 and K_2) is gradually reduced as an increasing number of surface residues are neutralized. This trend is reflected in the total free-energy change, ΔG_{tot} , for the binding of two Ca^{2+} ions (see Table 1): $\Delta G_{\text{tot}} = -RT \ln(K_1 \cdot K_2)$. For M0 this free energy change is -97 kJ mol^{-1} . Neutralization of one charge (as in M5, M6 and M8) changes ΔG_{tot} to about -90 kJ mol^{-1} with minor variation, depending on which charge is involved. Neutralization of two charges (M7, M9 and M10) leads to $\Delta G_{\text{tot}} = -83 \text{ kJ mol}^{-1}$ and for M11 with all three charges neutralized we find $\Delta G_{\text{tot}} = -77 \text{ kJ mol}^{-1}$. Thus, on average each of these three charges contributes -7 kJ mol^{-1} to ΔG_{tot} , the effect being additive within experimental errors (Table 1). For example, neutralization of Glu 17 (M5), Asp 19 (M6) and Glu 26 (M8) changes ΔG_{tot} by 8, 8 and 4 kJ mol^{-1} respectively, whereas the substitution of all three charges (M11) changes ΔG_{tot} by 20 kJ mol^{-1} . Furthermore, attempts to rationalize the Ca^{2+} affinity of EF-hand proteins exclusively in terms of the nature and spatial arrangement of groups directly involved as ligands are likely to fail because of the strong influence of surface charges. The possible extension of this statement to other ion-binding proteins should not be discounted.

Another parameter of importance to ion-binding proteins with two or more ion-binding sites is the free energy of interaction between the sites, $\Delta\Delta G$. In the present case, positive cooperativity ($\Delta\Delta G < 0$) is observed for Ca^{2+} binding in the wild-type and all mutant calbindins D_{9K}. A lower limit of $|\Delta\Delta G|$, $|\Delta\Delta G|_{\text{min}}$, can be obtained solely from the macroscopic binding constants as

$$|\Delta\Delta G|_{\text{min}} = RT \ln \left(\frac{4K_2}{K_1} \right)$$

(see Table 1). Calculated values of $|\Delta\Delta G|_{\text{min}}$ are listed in Table 1. For M0, $\Delta\Delta G$ has previously been determined as $-5.1 \pm 0.4 \text{ kJ mol}^{-1}$ (ref. 6).

We can thus state that, with the exception of M7, the cooperativity of Ca^{2+} binding increases as one or two negative surface charges are neutralized. A molecular explanation of this finding might be that neutralization of some of these charges diminishes the electrostatic repulsion of negative surface charges (for example Glu-51 and Glu-60) linked to site II. A closer approach of the two sites is thus facilitated and transmission of conformational effects on Ca^{2+} binding to one of the sites⁷ would accordingly be more efficient.

Finally, we can speculate on a possible biological role for these negative surface charges. A proposed function for calbindin D_{9K} is to facilitate Ca^{2+} transport through intestinal epithelial cells^{8,9}. The Ca^{2+} -ATPase of the enterocyte basolateral membrane pumps Ca^{2+} out of these cells. Interaction between

Table 1 Macroscopic binding constants (K_1 and K_2), free energy of binding of two Ca^{2+} ions ($\Delta G_{\text{tot}} = -RT \ln(K_1 \cdot K_2)$), and lower limit of the absolute value of the free energy of the site-site interaction ($|\Delta\Delta G|_{\text{min}} = -RT \ln(4K_2/K_1)$) of the wild-type (M0) and mutant calbindins D_{9K}

Protein	Neutralized side chains	$\log_{10} K_1$	$\log_{10} K_2$	ΔG_{tot} (kJ mol ⁻¹)	$ \Delta\Delta G _{\text{min}}$ (kJ mol ⁻¹)
M0	—	8.3	8.6	-97	4.7
M5	Glu 17	7.4	8.1	-89	7.7
M6	Asp 19	7.6	8.0	-89	5.8
M7	Glu 17, Asp 19	7.4	7.1	-83	1.7
M8	Glu 26	7.8	8.4	-93	6.4
M9	Glu 17, Glu 26	6.8	7.8	-83	9.2
M10	Asp 19, Glu 26	6.9	7.6	-83	7.8
M11	Glu 17, Asp 19, Glu 26	6.8	6.7	-77	2.9

$\log_{10} K_1$ and $\log_{10} K_2$ were obtained from titrations of the mutants with calcium in the presence of the chromophoric Ca^{2+} -chelator 5,5'-Br₂-1,2-bis(*O*-aminophenoxy)-ethane-*N,N,N',N'*-tetra-acetic acid (5,5'-Br₂ BAPTA; ref. 11) in 2 mM Tris-HCl buffer at pH 7.5 and 25 °C, as previously described⁶ for M0 in the presence of the analogous chelator Quin 2 (ref. 11; 2-[[2-bis(carboxymethyl)-amino]-5-methylphenoxy]methyl]-6-methoxy-8-[bis-(carboxymethyl)amino]quinoline). The data were analysed by a least-squares fit directly to the experimental quantity—the absorbance at 263 nm as a function of total calcium concentration⁶. The Ca^{2+} -binding constant of 5,5'-Br₂BAPTA was set to $1 \times 10^7 \text{ M}^{-1}$, as determined relative to that of Quin 2. $|\Delta\Delta G|_{\text{min}}$ was derived from the definition of the free energy of interaction between the two sites in a two-site protein, $\Delta\Delta G = -RT \ln(K_{I,II}/K_I) = -RT \ln(K_{II,I}/K_{II})$, where K_I , K_{II} , $K_{I,II}$ and $K_{II,I}$ are the microscopic (site-) binding constants as defined in Fig. 2. Because the titration method used here cannot distinguish between the two sites of the protein it yields only the macroscopic (stoichiometric) binding constants, K_1 and K_2 (see Fig. 2). Using the relations between macroscopic and microscopic binding constants ($K_1 = K_I + K_{II}$, $K_1 \cdot K_2 = K_I \cdot K_{II,I}$), we may rewrite the expression for $\Delta\Delta G$ as $\Delta\Delta G = -RT \ln \{(K_2/K_1) \cdot (1 + \eta)^2/\eta\}$, where $\eta = K_{II}/K_I$. Given that $(1 + \eta)^2/\eta$ has a minimum for $\eta = 1$ and that $\Delta\Delta G < 0$ for all proteins in this study, a lower limit of $|\Delta\Delta G|$ can be obtained from K_1 and K_2 as $|\Delta\Delta G|_{\text{min}} = RT \ln(4K_2/K_1)$.

calbindin D_{9K} and the basolateral membrane Ca^{2+} -ATPase has been demonstrated by Walters (ref. 10 and personal communication). As there is no evidence for any hydrophobic surface patches on calbindin, the interaction might occur through surfaces with complementary charges and have a double role. First, the proteins would be docked into a favourable position for Ca^{2+} ions to be transferred from calbindin to the ATPase. Second, the surface charges of both proteins would be partially neutralized as a result of the close approach of opposite charges. The Ca^{2+} affinity of the calbindin would then decrease, and that of the ATPase might increase. The net effect would favour Ca^{2+} transport from calbindin to the Ca^{2+} pump.

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Industrial biotechnology bonanza

Off-the-shelf genes, a 3,000-litre bioreactor and a fermentation monitoring system are just a few of this week's offerings for getting biotechnology products to market.

BACHEM Bioscience has a **cross-linking reagent** that it says it can use to link virtually any peptide to an appropriate protein carrier, such as bovine serum albumin or keyhole limpet haemocyanin, for antibody production (*Reader Service No. 100*). The reagent is the *N*-1-maleimido-6-aminocaproyl ester of 1-hydroxy-2-nitro-4-benzene-sulphonic acid (mal-sac-HNSA). Bachem uses the water-soluble, heterobifunctional crosslinker in its custom services to conjugate a peptide amino group with a carrier cysteine or a peptide cysteine residue with a carrier amino group. A reaction with amino groups releases the dianion phenolate, HNSA, which allows spectrophotometric quantitation of the reaction in progress. Bachem says conjugates of peptides to appropriate carriers have elicited peptide-specific antibodies with no detectable antibodies specific to the crosslinker.

Cellon Sarl sells **reusable microcarrier beads** for culturing anchorage-dependent cells (*Reader Service No. 101*). The company says its Bioglas glass microcarrier



Cellon Sarl says its microcarrier beads can be reused up to 15 times without compromising cell growth.

beads can be used 15 or more times without a reduction in cell growth. The beads can be used in cell cultures of up to 40 grams per litre, at densities of up to 5×10^6 cells per millilitre. Cellon Sarl says viable cell recovery levels of between 95 and 99 per cent can be achieved because of the short trypsinizing times required. The Bioglas beads are neutrally buoyant in culture media, and are produced in a range of sizes and specific gravities.

British Bio-technology Ltd has added several new **off-the-shelf genes** to its Designer Genes line (*Reader Service No.*

102). The company now has available genes for β -endothelial cell growth factor, acidic fibroblast growth factor, basic fibroblast growth factor, transforming growth factor α , transforming growth factor β and interleukin 1 β (natural). BBL says β -ECGF is an acidic polypeptide which is mitogenic for endothelial cells and fibroblasts *in vitro* and angiogenic *in vivo*. The exact *in vivo* role of the fibroblast growth factors is still unclear, but BBL says acidic FGF has been shown to be identical to, or related to, other growth factors including ECGF, EDGF II, BDGF and AGF 1. Basic FGF is similar or identical to EDGF I, HGF, CDGF and AGF 2, among others, says the company. BBL sells the above genes cloned in the polylinker of pUC18 for £1,900–5,100 (UK). The IL-1 β gene is supplied cloned in the polylinker of M13mp18.

Culture media

Mediatech has recently published a new **catalogue** covering its Cellgro line of culture media (*Reader Service No. 103*). The free 35-page catalogue lists Mediatech's standard information on product formulations and quality control, and introduces several new products, including trace element concentrates and a medium with low serum level requirements. The catalogue also describes a new non-toxic 25-litre capacity flexible bag for large volume media users, and Mediatech's experimental Media Pool, which helps users to



Mediatech's latest catalogue on its Cellgro line of cell culture media lists a host of new products.

pool their special media requests to save money.

The Scientific Products division of Baxter Healthcare Corporation has recently announced that it will be distributing **tissue**



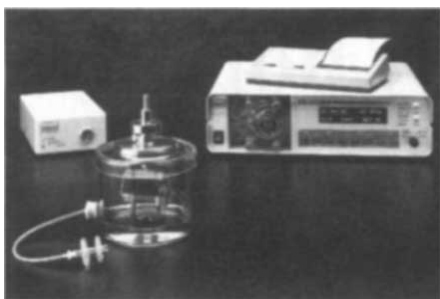
New culture products from Baxter: custom preparations, freezing media and enzyme-free dissociation solution.

culture media products for Specialty Media, Inc (*Reader Service No. 104*). Scientific Products is now offering custom-formulated tissue culture media prepared to the specifications of the customer in volumes greater than one litre, and cell culture freezing media for the cryopreservation of tissue cells. The company is also selling several mammalian cell transfection products and an enzyme-free cell dissociation solution as a replacement for trypsin. Baxter says the cell dissociation solution contains no proteins and does not remove cell surface proteins.

Novo Biolabs offers a variety of antibiotics, growth factors, hormones and cell culture enzyme preparations for **cell and tissue culture** (*Reader Service No. 105*). Gentamycin, kanamycin and penicillin are among the company's range of antibiotics for the control or elimination of microbial and fungal contaminants. Growth factors and hormones available from Novo BioLabs include epidermal cell and platelet-derived growth factors, porcine and bovine glucagon crystals, and insulin. For disaggregating tissue and dissociating cell monolayers while maintaining cell viability, Novo BioLabs offers a range of trypsin of porcine and bovine origin and its NovoZym cell wall lysing enzyme, which is assayed for solubility, loss on drying residue or ignition, and *in vivo* activity.

Culture containers

Liveco Biomechanical Instruments has an experimental system named Vitrodyne for the application of **controlled mechanical forces** to cells, tissues and organs main-

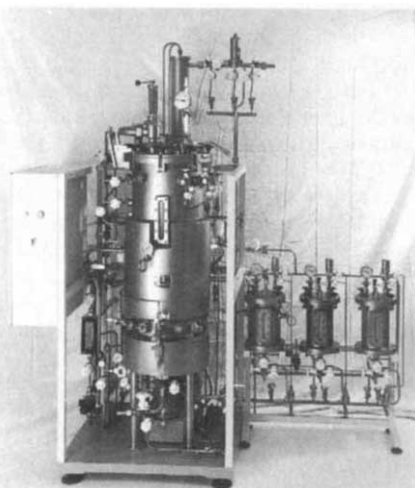


The Vitrodyne from Liveco flexes and stretches tissues in culture.

tained in sterile culture (Reader Service No. 106). The heart of the \$9,800 (US) Vitrodyne is a sterilizable pneumatically driven forcing frame suspended in a glass culture vessel. A computerized controller module located outside the incubator continually monitors and executes pre-programmed static or dynamic force profiles in either tension or compression modes. The system has proportional feedback circuitry which allows the force to be controlled with a resolution of 10 mg. Liveco says the Vitrodyne can be used in blood vessel mechanics and biochemistry, bone growth and remodelling, soft tissue repair under static or dynamic loading, and

studies of the morphogenesis and biochemistry of muscle, tendon, ligament, skin, and cartilage in response to mechanical stress.

LH Fermentation Ltd has introduced its P800 range of fermentation systems for the **larger capacity fermentation** market (Reader Service No. 107). The systems are available with volumes of between 80 and 3,000 litres, and are customized for each application. A wide range of options and vessels are available, which may be inter-linked to build multiple fermenter systems including seed, harvest and medium make-up vessels. Fully contained systems



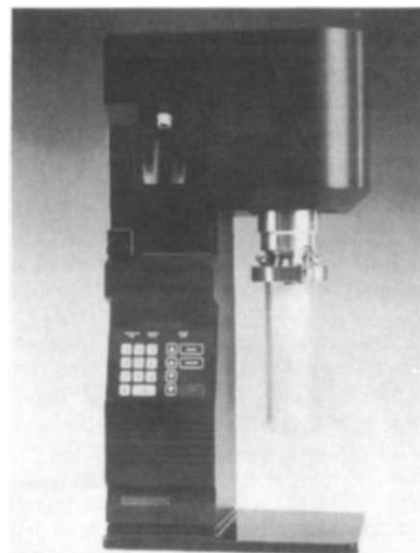
From LH Fermentation's P800 line of fermenters.

and upstream and downstream processing facilities are available, and the systems are computer compatible for automated supervisory control and data management.

The Waters division of Millipore has developed an **online fermentation monitoring system** for the aseptic filtration, analysis and monitoring of fermentation broths and tissue culture fluids (Reader Service No. 108). The so-called FAM fermentation monitoring system combines membrane filtration and HPLC analysis to produce an automated composition sensing device for the continuous monitoring of nutrients, metabolites and products in a bioreactor. The FAM system can be set for continuous or periodic sampling, and the sampled broth can be returned to the culture vessel. Waters says the \$4,761 (US) system can measure more components, faster and more accurately in less time than it takes to assay one component by traditional techniques.

Separation science

The Benchmark **rotary biopurification system** from Membrex, Inc is a compact, laboratory benchtop system which combines a proprietary membrane filtration material with rotary separation technology (Reader Service No. 109). Membrex says the hydrophilicity, chemical stability



Rotary biopurification system from Membrex.

and physical durability of the membrane used in the Benchmark make it suitable for a broad range of biological separations. Its rotary process reduces shear rates and improves fluid dynamics for separation efficiency, says the company, and its ability to prevent fouling provides high product yields and fast throughput. The UltraFlic membranes used in the Benchmark system are available in molecular weight cutoffs between 10,000 and 1 million daltons, and are autoclavable. The \$6,250 (US) Benchmark system can be used to filter small amounts of sample in batches, or to filter large sample volumes using a peristaltic pump.

Romicon, Inc is now distributing TosoHaas **large-scale chromatography** products (Reader Service No. 110). Romicon says the TosoHaas Protein Prep LC system for pilot- and production-scale liquid chromatography can deliver performance comparable to lab-scale HPLC equipment. For high speed and resolution applications, Romicon recommends pre-packed TSK-GEL columns, which have the identical chemistry of the TSK materials used for analytical purposes for use with the system. The TSK-GEL columns range in size from 55 to 210 mm i.d., and come in lengths of 20, 30, 40 and 60 cm. Romicon sells Toyopearl packed columns for applications where economy and performance are important. Toyopearl is an acrylic-based material which can withstand pressures up to 100 p.s.i. and can operate at linear velocities of up to 400 cm per hour. The Toyopearl columns come in volumes of 0.5 to 170 litres. □

These notes are compiled by Carol Ezzell from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.

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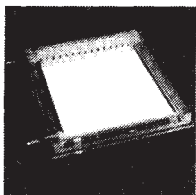
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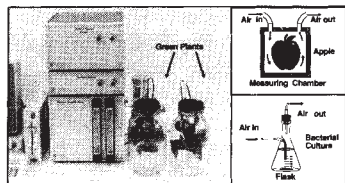
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